SEM based comparative study of diluted pharmaceutical agents silica and potassium chloride

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Abstract

The morphological changes occurring because of trituration and solid dilution have been assessed by surface electron microscopy to follow medicinally active ingredients and validation of these findings became the objective of the study. Diluted Pharmaceutical agents Silica and Potassium Chloride were analyzed for comparison. The SEM presentation of the biochemic drugs support the idea that it is the unique characteristic change of the virgin drug substances produced because of trituration and solid dilution which is responsible for the changes in medicinal properties. The high intensity electron beam caused multiple fractures on softer molecules which helps distinguishing between lactose and the drug particles.

Keywords: Silica, Potassium Chloride, Sugar (Saccharum Lactis, U. S. P.), Diluted Pharmaceutical Drugs, Phase difference, Nano particles

Introduction

The paradigm shifts of different medical systems, drug used in different concentrations, unavailability of 'evidence base' of serially ultra-diluted drug beyond 'a placebo', failure to detect medicinally active ingredient in a ultra-diluted drug were the motivating factors, and validation of these findings became the objective of the study. The hypothesis related to the use of the drug in concentrations, as used in homeopathic system of medicine vis-à-vis known relevant information of the drug available especially in modern medicine and science has been put to test in this study. Considered that the surface electron microscopic assessed morphological changes occurring because of trituration and solid dilution may provide clues.

Silica particles, chemical structure shown in Figure, are widely used in our daily lives owing to their many attractive, excellent physicochemical, mechanical and optical properties. Silica nano particles (SNP) have revolutionized science by its widespread application in electronics, sensor technology, purification, agriculture and medicine. Most commonly silica particles were blended with cosmetic talcum powders since ancient times. Silica is most widely used as desiccant in industries and homes. At present, silica nanoparticles are extensively studied for their potential as photonic crystals [Rahman et al 2009], chemical sensors, biosensors [Rong et al 2013], nano-fillers for advanced composite materials, markers for bioimaging, substrate for quantum dots and catalysts etc. In medicine silica particles have been tried as excipient in various drug formulations with encouraging and presently acceptable results. The variation of properties with the particle size is widely studied for nano scale metals and semi-conducting materials (Slowing et al, 2008). The most commonly investigated silicon-based materials for drug delivery are porous silicon and silica, or silicon dioxide (Torney et al 2007). The nano-architectures include calcified nanopores, platinum-containing nanopores, porous nanoparticles, and nanoneedles. The density and diameter of the nanopores can be accurately controlled to achieve a constant drug delivery rate through the pores [Demangeat, 2010]. Examples of therapies being investigated for use with silicon-based delivery systems include porous silicon embedded with platinum as an antitumor agent, calcified porous silicon designed as an artificial growth factor, silicon nanopores for antibody delivery, and porous silica nanoparticles containing antibiotics, enzymes, and DNA [Navarro, 2008]. Silica is commercially available and also abundant in nature, found in apples, cucumbers, raisins and strawberries.

In Homeopathic Medicine Silicea derived from silicon dioxide, is a biochemical drug sold in tablet form, as well other potencies as 3c, 6c, 200c, or so available commercially. Terms Silicea, Silica, and Silicon Di Oxide have the same meaning in the article. It has been used by homeopathic practitioners for various disorders like chronic inflammatory lesions, healing of wounds, removal of minute foreign bodies embedded in mucous membrane, cysts, etc.

Ultra diluted (UD) drugs are now being extensively studied for ascertaining their evidence base (Sharma et al, 2010). Sufficient exploratory research work has not been conducted on structure of homeopathic-biochemical diluted drugs (HBD). The HBD are prepared by diluting the mother drug substance with vehicles like ethyl alcohol, water or lactose / Sugar of Milk in 1:99 (denoted level of dilution with a suffix 'c' e.g. 30c) or 1:9 (denoted level of dilution with a suffix 'x' e.g. 12x) and then subjected to physical grinding, triturating, pulverization or succession as required by the relevant procedures provided in pharmacopoeia of

different countries like India, Britain, America and more. The mother substance of UD disintegrates to contain particles of inconsistent size including nano particles. The drug particles reduce in size gradually because of each subsequent serial dilution and trituration like processes involved and in some cases they are of nano particle size, that is in the order of 1 to 100 nanometers (1 micrometers = 1000 nm), with increased solubility. It is believed that trituration, pulverization, or grinding enhances medicinal influence of the substance. Ethanol, water, Ammonia, sonication or vibrations play an important role in preparation of nano particles [Elia and Niccoli, 1999]. The chemically pure drug particles are triturated with sugar lactose. With each successive dilution with lactose the quantity as also the concentration of drug reduces. Physical characteristics like size, shape, smoothness, solidity, surface, substitution, surrounding, similarity, stiffness, electrical gradient, optical properties, memory of the drug particle, temperature are some of the features that possibly determine the property of the ultradiluted drugs prepared by successive dilution [Chaplin, 2007 & Elia et al, 2007]. Usually cellular uptake occurs when the molecular size ranges from 5 to 250 nm both in animal and plant cells. To trigger biological response metabolism is not essential, only presence in the vicinity of the cell can initiate a response [Ye Y et al 2010].

In the present SEM study attempt has been made to compare the morphology and other associated characteristics of Silicon dioxide (Silica, Silicea), a laboratory chemical, with various potencies of biochemical drugs Silicea (derived from Silicon dioxide), Kali Mur (Potassium Chloride, KCl), and Lactose Sugar (Saccharum Lactis, U. S. P.) $C_{12}H_{22}O_{11}+H_2O$, mw 359.16.

Kali Mur (KCl), and Silicea have important medicinal values. The medicine grade chemicals Lactose, Kali Mur (KCl), and Silicea used for comparative study with the respective biochemical drugs. The 6X, 12X, 30X, 200X potencies of Silicea were used for this comparison. The potencies of Kali Mur used were 6X, 12X, and 30X. Presence of Lactose was studied as it was used for triturating the biochemical drugs.

Materials and Method

All the drugs and chemicals used for this comparative analysis were procured from the local authorized drug stores of Hahnemann Publishing Company. Silicea and Kali Mur of 6 X, 12 X, and 30 X potencies of each drug were of HAPCO and Boerick & Tafel, manufactured in June 2006. Laboratory/ Pharmaceutical grades Silica (SiO_2), Potassium Chloride (KCl) used for the comparative study were manufactured by Merck. All chemicals and drugs were used as received from the vendors without further purification. The drug and the chemical samples were desiccated, and vacuum dried for the study. Chemical structure of Silicon dioxide is given in Fig. 7.

Chemical drying was avoided for risk of contamination. The non-conductive samples were sputter-coated with gold of optimum thickness for attaining reasonable conductivity. KCl, an odorless metal halide, Molar mass: 74.5513 g/mol, Refractive index (n_D): 1.4902 (589 nm), is used as medicine (e.g. low blood pressure), food processing and in judicial execution through lethal injections and scientific applications.

JEOL JSM 5800 Scanning Electron Microscope was used for the surface and interface characterization of the experimental drugs and chemicals. The equipment had the following specifications: Acceleration voltage: 0.3 - 3kV (100V Steps), 3 - 30 kV (1kV Steps) Electron gun: W Filament; Working distance: 8–48mm. The SEM images of the study materials were obtained at suitable and selective magnifications of 50, 100, 250, 500, 3000, 4000, 5000 and 7000.

Result and Discussion

Fig. 1 contains the magnified SEM studies of Silica which is suggestive of heterogeneous clean pattern, with sharp irregular voids and phase difference as shown in Fig. 1 A, B, and C. With 500x magnification of the Silica sample, a clean definite geometrical pattern becomes distinct. In between these particles, voids and some phase difference seems to be present. The depressed surfaces with irregular boundaries having "sprinkled salt" appearance because of unidentified particles could be noticed. Margins bombarded with electrons resulted in scattering, which illuminated the adjacent area to display angle between flanking surfaces and the structural robustness. Size of these irregular portions of Silica particles are roughly 117 x 100 x 50 µm and 68 x 50 x 100 µm.

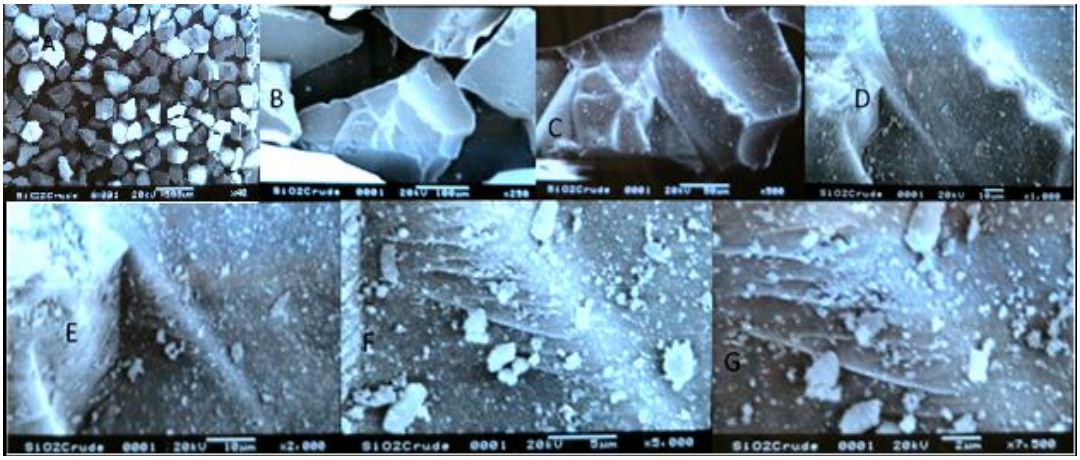

Fig. 1: SEM images of Silica (SiO_2) at magnifications: A. x 40, B. x250, C. x500, D. x1000, E. x2000, F. x5000, and G. x7500

The 1000x magnification of Silica is primarily suggestive of two types of morphology having some void; as a result the phase continuity is lost. The crevice like appearance between the two portions of Silica particle becomes prominent. The ridge in the adjoining area seemingly separates the two portions. Because of electron scattering, the undulated rough margins on top show bright area. This also indicates robustness of the Silica particle. However, in some cases the presence of well-separated structures is evident, which can be distinguished easily, suggesting the lack of phase adhesion. The sprinkled rod and triangular shaped particles of nm to µm size, probably debris, are distinctly identifiable.

At 2000x magnification, Silica is primarily suggestive of the two lamellar rock-like stacked structures of Silica placed at about 90^0 to each other in the inner part of the ridge and adjacent block whereupon debris like structures are scattered. Stalked lamellar size is about 40 µm x 10 µm, while each lamella is of about 0.5µm thickness and 12 lamellae could be seen on one side. From the oblique ridge a number of prominent lamellar projections at about 90^0 can also be seen, as if a wedge shaped portion is broken off the Silica particle.

In 5000x magnification of Silica, Fig. 1F, the phase difference between the two structures is not present. The phase seemingly appeared as electronic beam shadow of the vertical structure. Shadow under the small fragments type structure could also be seen. The firm and distinct structure, with irregular geometrical pattern, sharp margins, seems to be projecting like a shelf. Small numerous particles appear to have been kept chaotically in the shelf. These small irregular sized granules, varying from 0.12 µm to 0.625 µm seems to be detached part of bigger particles of the size 3.125 µm x 2.5 µm or so.

At magnification 7500 x of Silica, Fig. 1G, the 0.0909 µm thick shelve like structures, forming gaps of the size 0.909 µm to 2.36 µm, showing large surface area which may allow free passage of less than nano sized particles, liquids, and dissolved materials through them. This shelves set up may cause (either one or collective) physical, biochemical, and pharmaceutical phenomenon like entrapment, adsorption, absorption, adhesion, capillary action etc. resulting in filtration at a slow rate [Elia and Niccoli, 1999 & 2004].

The SEM study of Silicea 6X Biochemic tablet is suggestive of clean inhomogeneous pattern, with sharp irregular voids and phase difference as shown in Fig. 2 (a – e). With 1000x magnification of the Silicea 6X sample, as shown in Fig. 2 a, clean definite geometrical patterns become distinct. In between these structures, voids and some phase difference can be seen. There is a variation in density of the inclusions. On soft masses tear of the size of about 20 µm x 30 µm and 16 µm x 16 µm because of less resistance to the impact of electronic beam may be noticed. In comparison to the corresponding SEM image it is clear that this portion is not a part of Silica. About 3 – 5 pits of the size 1µm x 1 µm are present on these soft masses. In Fig. 2 b. a central dark zone of about 6 µm x 7 µm is seen. The smaller dense particles with bright margins seem to be Silicea particles. Size of these irregular portions of Silicea particles are roughly between <1µm and 10µ.

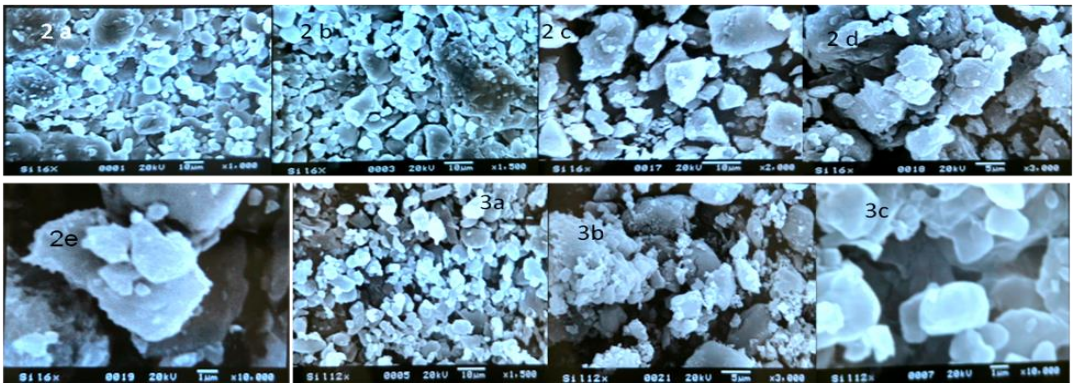

Fig. 2: SEM images of Silicea 6X Biochemic tablet at magnifications 2a. x1000, 2b. x1500, 2c. x2000, 2d. x3000 and 2e. x10000

Fig. 3: SEM images of Silicea 12X Biochemic tablet at magnifications 3a. x1500, 3b. x3000, and 3c. x10000

In Fig. 2 c, at x2000, the biggest triangular particles, at the center of the frame of the size 10 µm x 15 µm x 12 µm with brightness at the margins are probably Silicea fragments and smaller similar shaped particles are also fragments of Silicea. Bigger masses comparatively looking dull having fractured surface are part of lactose. In this frame phase difference is evident and suggesting the lack of phase adhesion.

In Fig. 2 d, at x3000 firm and bright particles can be distinguished with the fractured soft masses which do not have bright margins. The fractured mass with dull whiteness, of comparatively large longitudinal structure is seemingly lactose have phase adhesion in its long axis. Few comparative small bright particles of the size 4.66 x 1.6 µm at or near the margins of the frame are likely to be a Silicea fragment. Phase adhesion at the background can be seen in the left central portion of the frame whereas at other corners phase difference is evident. A sharp horizontal or oblique slit, which is not a fracture, appears on the harder particle, seemingly Silicea, and is marked with the arrow.

In Fig. 2 e, at x10000, a chunk of thin central mass having phase adhered small particles agglomerated and stacked together are probably part of Lactose. Comparatively very small scattered particle, e.g. at about 1 and 2 o'clock position, are probably of Silicea. Phase difference can be distinguished in this frame.

The 1500x magnification SEM image of Silicea 12X, Fig. 3a, of trituration is suggestive of primarily two types of morphology having some void; as a result the phase continuity is lost. A mixed mass of Lactose and Silicea 12x is in-homogenously scattered. At 3000x, Fig. 3b, the stacked and agglomerated portion between 8 to 11 o'clock positions is a mass of Silicea with smaller fragments scattered all around. These stacking have structure of 10 µm thickness, which is similar to SEM image of pure Silica. In Fig. 1e, the stalked lamellar thickness is about 10 µm. Distinct fractured soft masses of dull brightness, shown with the arrows, at about the central position of the frame are likely to be of Lactose. It may be interpreted that the Silicea particles have moved on to the surface while the soft lactose mass gets underneath.

The 10000x magnification SEM image of Silicea 12X, Fig. 3c, of trituration is suggestive of primarily two types of morphology having some void; as a result the phase continuity is lost.

Comparative study of Silicea 6x and Kali Mur 6x

Description above of Fig. 2 b, is related to Silicea 6x, and Figure 4a shows SEM magnification of Kali Mur 6X, at x1500. While comparing the SEM magnification of images at x1500 of Silicea 6x and Kali Mur 6x, it is seen that white cuboidal structures, marked with arrows, of Kali Mur are distinctly recognizable. While Silicea 6x particles are non-agglomerated, the Kali Mur 6x particles of irregular sizes and dimension are scatteredly adhering to form clusters. Some pithole type Kali Mur particles are also seen. In between there is a large void distinctly noticeable with the phase difference. This may help in assesing quality of the Biochemic drugs in various dilutions.

Fig. 2e describes the x10000 image of Silicea 6x. In Figure 4c at x10000, a chunk of central mass having phase adhered about 7 -8 small particles agglomerated and stacked together are of Kali Mur 6x. The smooth rounded, geometrical shape is the characteristic presentation of Kali Mur. Comparatively fractured mass at the base in dark is of Lactose. Phase difference can be distinguished in this frame. The characteristic appearance of agglomerated mass can be considered as a signature of Kali Mur. The absorbance of electronic beam illuminates, making Kali Mur particles distinctly identifiable in all its biochemical dilutions.

Comparative study of Silicea 12x and Kali Mur 12x

At 3000x, Fig. 5a, another view of the stacked and agglomerated portion between 8 to 11 o'clock positions is a mass of Silicea 12x with smaller fragments scattered all around. Descriptions, similar to the image are given along with the Fig. 3b. Fig. 5b, is the image of

Kali Mur 12X x3000. It shows the characteristic agglomeration of Kali Mur, although sparse, because of dilution. The margins of Kali Mur particles are smooth and shiny where as the particles of Lactose are irregular, saw toothed, and non adherent. The large void between the diluted & triturated drug particles makes the deeper view clear. The large dark particles at the base show fracture at places, typically indicative of Lactose, the vehicle used for dilution [Demangeat, 2010].

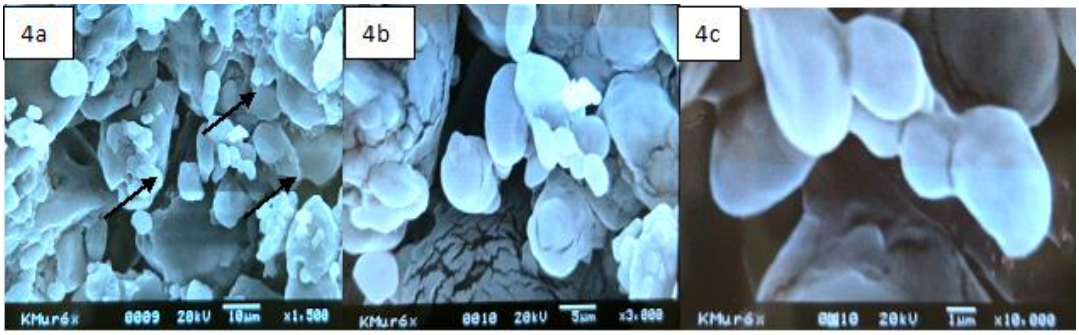

Fig. 4: SEM images of Kali Mur 6X Biochemic tablet at magnifications 4a. x1500, 4b. x3000 and 4c. x10000

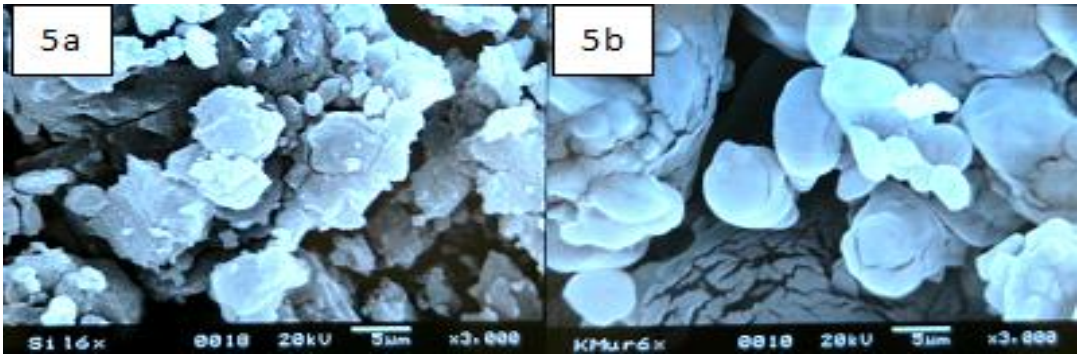

Fig. 5: SEM images of Silicea 12x and Kali Mur 12x at x3000 magnifications

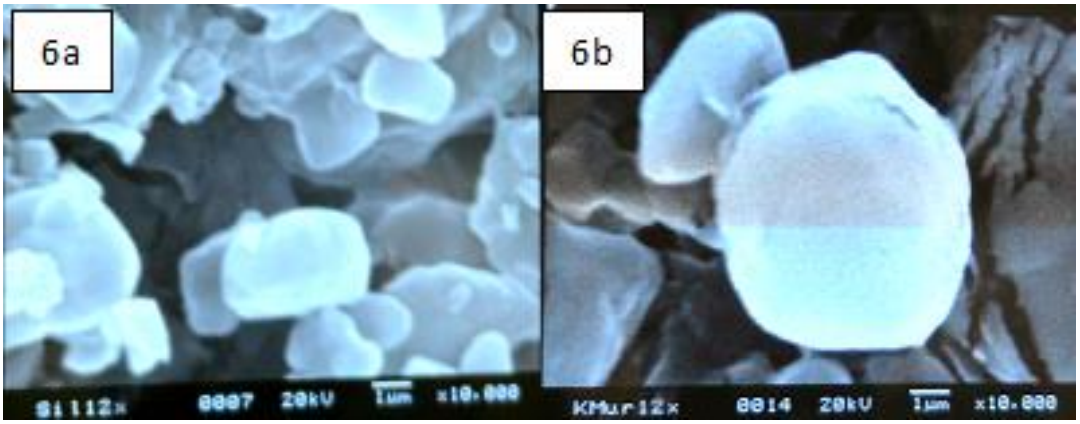

Fig. 6: SEM images of Silicea 12x and Kali Mur 12x at x10000 magnifications respectively

The 10000x magnification SEM image of Silicea 12x, Fig. 6a, of trituration is suggestive of primarily two types of morphology having distinct void; as a result the phase continuity is lost. The sharp outlined Silicea 12x particles are scattered as non-agglomerated, non-adherent particles of varying size. Each particle has distinct geometry resembling that of Silicea, mostly they are somewhat cuboid. The underneath fractured lactose can be seen distinctly. The borders of Silicea particle at 12x are shiny because of the incident electronic beams. In this Fig. lactose could be noticed on the top surface of the Silicea particle. The large mass of Kali Mur 12x at x10000, as shown in Fig. 6 b, is adhering to smaller particles just beneath. The phase difference further distinguishes the linearly fractured vehicular structure. The high intensity electron beam has caused multiple fractures on it. Probably the tangential electronic beam have put dents on the surface of Kali Mur 12x.

In general, it may be noticed that the absorbance of electronic beam illuminates, making Kali Mur particles distinctly identifiable in all its biochemical dilutions.

The density and diameter of the nanopores can be accurately controlled to achieve a constant drug delivery rate through the pores. Porous hollow silica nanoparticles have unique architecture of having parallel pores which may allow various physical-chemical actions. It may be filled with suitable drugs for controlled release and provide opportunities for designing zero premature release systems, which could be operated under the control of various external physical or chemical stimuli [Iris et al 2012].

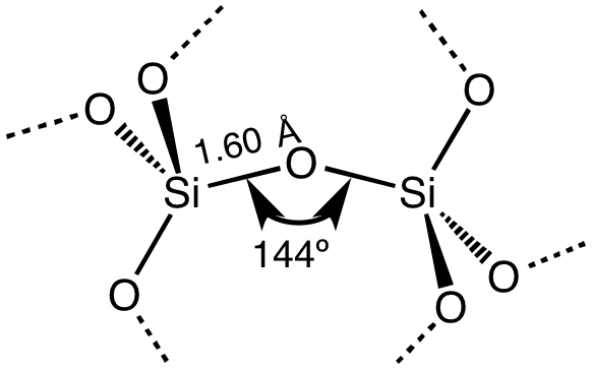

Fig. 7: Chemical structure of Silicon dioxide

Conclusion

The globular Kali Mur particles have unique characteristic shape even on very high dilutions, which may allow adherence to specific sized and structured surface.

The Biochemical solid dilutions are different than liquid dilution in making the medicinal substance resurfacing to initiate suitable action on dissolution of the diluent in the system. The amount, shape and size of the biochemic drug particles determine the rate of reaction depending on the suitable environment.

The SEM presentation of the biochemic drugs support the idea that it is the unique characteristic change of the virgin drug substances produced because of trituration and solid dilution which is responsible for the changes in medicinal properties. The high intensity electron beam caused multiple fractures on softer molecules which helps distinguishing between lactose and the drug particles.

Study Limitations

This study has several limitations. It is laboratory based assessment and, therefore, it has all the pitfalls of such analysis. There are some other components, which are naturally present with Silicea and Kali Mur for its different activity. Not all the dilutions of the medicines are taken into consideration. For confines of the research work non-parametrical statistical analysis along with the clinical applications were not conducted. Medicinally active ingredients of HBM are difficult to detect analytically in Laboratory. HBM is not detectable in biological specimen. Commercially available medicines were only tried. Inference could be drawn about the commercial quality of the manufactured drug, since that is beyond the purview of this dissertation the same is not included.

Conflict of interest

There is no conflict of interest.

Acknowledgement

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