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Standardization and analytical evaluation of novel siddha formulation *Singi chenduram*: An approach towards nano drug delivery system

K. Rajamaheswari*, M. Pitchiah Kumar, V. Banumathi and S. Sudharsan

Post Graduate Department of Gunapadam (Pharmacology),Government Siddha Medical College, Arumbakkam, Chennai, Tamil nadu, India

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ABSTRACT

Standardization of siddha preparations is of utmost important task in establishing the active components of the drug responsible for its biological activity. The World Health Organization (WHO) guideline on evaluating the physiochemical property of a siddha formulation offers a greater value of Indian formulation in global market. Characterization of siddha formulation renders wide range of information in predicting the nature and structure of phytoconstituents which renders the actual therapeutic efficacy of the formulation. Even centuries before Indian systems of traditional medicines like siddha are the pioneers in developing nano drug delivery to the target site. The main aim of the present study is to prepare nano size formulation Singi Chenduram (SC) and also characterize the same by using sophisticated techniques like Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). The results obtained from the SEM analysis shows that most of the particles present in SC are in nano and near nano size ranges from 71.32nm – 95.20nm.Further results obtained from the TEM study clearly projects that all the particles present in aggregates of SC formulation are within the size range of 50nm-200nm.This results shows that the formulation SC contains nano size particle which claims for its efficacy against the dreadful infectious disease like tuberculosis. In conclusion data's retrieved from the current characterization study of SC confirms the size, shape and nature of nano-components of SC and hence this could be used as standards for evaluating quality and reproducibility of the Singi Chenduram in future.

Keywords: *Singi Chenduram*, Siddha formulation, Standardization, Nano drug delivery, WHO.

INTRODUCTION

Herbs and herbo minerals are the major components in all indigenous preparations of traditional medicine and common element in siddha, ayurveda, naturopathic and native medicinal Indian herbal medicine emphasizes prevention of disease, rejuvenation of our body systems and it extends the life span and makes healthy life in balance and harmony. According to the recent estimate The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment for the treatment of various chronic disease [1, 2].

Siddha system of medicine is pioneers among other traditional therapies belong to south East Asia especially in treating dreadful infectious disease like tuberculosis. Formulations have been prepared as per Vedic literatures and processed for emphasizing tis efficacy before administering the same for clinical use. Metals and minerals play a vital role in most of the siddha preparation and because of this known reason usage of certain mercurial preparation

are banned in most of the foreign countries. Actually all the metallic preparations are detoxified with prescribed technique and level of metals has been dropped down to the acceptance range as recommended by the WHO guideline.

Rasa chenduram and *Lingha chenduram* are mercurial preparations used widely for various ailments. It was also found that most of the herbs in *Kandhaga rasayanam* a classical siddha preparation which in clinical practice for more than 50 years has anti-tubercular property [3]. Chenduram like *Aya chenduram, Kantha Chenduram, Velli Chenduram, Armugha Chenduram, Ayakantha Chenduram* are good haematincs. *Annabedhi chenduram* and *Vediannabedhi chenduram* are more commonly used and best haematinics [4].

'*Sayam'* is a term described in Siddha medicine which could be correlated with Tuberculosis. *Sayam* begins with vitiation of apana vayu in the large intestine. Vatham eventually overflows into circulation and relocates to the respiratory system. Additional thodams may mix with vatham or become dominant in its Pathology. Here all the Sapta Dhatus are involved in the manifestation of Rajayaksma, where all Dhatu are in Kshayavasta. The symptoms of Rajayakshma is differs according to the involvement of Sahasa (by excessive stress and strain) Vegasandarana (suppressing the natural urges), Kshaya (diminishing if dathu) Vishamashana (opposite to dietary regimen). The manifestation of Rajayakshma by Kshaya takes place by two different pathways according to the direction in which depletion of Dhatus takes place [5].

Nanotechnology provides the tools and technology platform for the investigation and transformation of biological systems, and biology offers inspiration models and bio-assembled components to nanotechnology. Nanobiotechnology is defined as a field that applies the nanoscale principle and techniques to understand and transform bio systems (living and non-living) and which uses biological principles and materials to create new devices and systems integrated from the nanoscale [6]. It is widely believed that formulation contains nano particle will effective bind with the bacterial membrane and aids in penetrating the cellular component of the organism thereby preventing its replication.

Singi chenduram is indicated as a wonderful drug for *Sayam* in *Yaakoebu loga chenduram 300*. There is no scientific validation behind this formulation. However it is possible to generate a data based evidence to standardize this medicine with reference to the authentic drugs. In view of standardizing *Singi Chenduram* (SC) a unique siddha formulation, the present work has been planned to formulate SC and carry out the analytical validation through SEM and TEM.

MATERIALS AND METHODS

Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDX) [7,8]

Undefined Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) is the best identified and most widely-used of the surface analytical techniques. High resolution images of surface topography, with excellent depth of field, are formed using a highly-focused, scanning (primary) electron beam. The primary electrons pierce a surface with an energy of 0.5 – 30 kV and produce many low energy secondary electrons. The intensity of these secondary electrons is mainly governed by the surface topography of the sample. An image of the sample surface can thus be constructed by measuring secondary electron intensity as a function of the position of the scanning primary electron beam. High spatial resolution is possible because the primary electron beam can be determined to a very small spot (<10 nm). High sensitivity to topographic features on the outermost surface (< 5 nm) is achieved when using a primary electron beam with an energy of < 1 kV. The SEM analysis was carried out in metallurgy department, IIT madras.

Transmission Electron Microscopy [9].

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 hold a standard size of grid upon which the sample is placed or a standard size of self-supporting specimen. Standard TEM grid sizes are a 3.05 mm diameter ring, with a thickness and mesh size ranging from a few to 100 μm. The sample is placed onto the inner meshed area having diameter of approximately 2.5 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 variety of designs of stages and holders exist, depending upon the type of experiment being performed. In addition to 3.05 mm grids, 2.3 mm grids are sometimes, if rarely, used. These grids were particularly used in the

mineral sciences where a large degree of tilt can be required and where specimen material may be extremely rare. Electron transparent specimens have a thickness around 100 nm, but this value depends on the accelerating voltage. The as prepared sample is mixed with ethanol and ultrasonicated further for dispersion of herbo medicine SC in the solvent. Few droplets of the solution is dispersed in the grid and it inserted into the TEM vaccum chamber to record the images. Magnification of the sample is a crucial technique to manage the sample for analysis. The TEM analysis was performed in IIT Madras.

RESULTS

A standard operative procedure involved in the preparation of *Singi chenduram*, along with certain analytical methods helps to reveal the chemical composition of formulation with their concentration and also ensure safety limits and accuracy of drug. In the present era in order to establish the safety concern the prepared drug should be understood well and interpreted with the help of modern technology and must be supported by proper scientific validation.

Undefined SEM in addition to low energy secondary electrons, backscattered electrons and X-rays are generated by primary electron bombardment. The intensity of backscattered electrons can be linked to the atomic number of the element within the sampling volume. Hence, some qualitative elemental information can be obtained.

The results of SEM analysis is illustrated in Figure 1 under section A,B,C and D. Figure 1A shows that overall particle size distribution of the sample SC in which most of the particles are in nano size range. Further in-depth analysis of SC on Figure B and C reveals that the segregated particles vary from minimum size of 71.32 nm to maximum of 95.20 nm. Similarly the Figure 1 D reveals that particle distributed from the size of 75.21nm to 95nm.
Transmission electron microscopy (TEM) is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed 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 such as a CCD camera.

Results obtained from the TEM analysis of the study drug SC is well illustrated in the Figure 2 under section A,B,C and D with the scale value of 200 nm, 50 nm, 100nm and 200 nm. The macro particle as well as nanoparticle observed in the sample. In the image B, it was observed that the smallest particle with size below 50 nm are bounded together. Similarly in TEM images of section D shows that the size range of particle is around 40-50 nm. Hence, the TEM result confirms the spherical shape and aggregated particle morphology obtained for the formulation SC.
After successive calcinations much smaller particle sizes are reached. Ultimately particle sizes reach colloidal scales (from a few microns to a few nanometers) these sizes help in better absorptivity and so the drug delivery is enhanced. In each cycle of calcinations the oxidation process would progress in a different way and various organo metallic complexes are formed intermediately before finally achieving desired pure metal oxide or sulfide.

TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons. This enables the instrument's user to examine fine detail even as small as a single column of atoms, which is thousands of times smaller than the smallest resolvable object in a light microscope. TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. TEMs find application in cancer research, virology, materials science as well as pollution, nanotechnology, and semiconductor research.

At smaller magnifications TEM image contrast is due to absorption of electrons in the material, due to the thickness and composition of the material. At higher magnifications complex wave interactions modulate the intensity of the image, requiring expert analysis of observed images. Alternate modes of use allow for the TEM to observe modulations in chemical identity, crystal orientation, electronic structure and sample induced electron phase shift as well as the regular absorption based imaging.

DISCUSSION

Siddha system is one of the most conservative medical systems in the world. In the field of medicine Siddhars had enlightened the world to save the human lives from various refractive diseases. In Siddha system, the medicines are not only made up of an herb which includes minerals, metals and other products of various organisms also.

Siddha medicines are always formidable because there are so many thaumaturgical medicinal preparations. Siddhars made some regime especially in the field of Pharmacology. In order to prove that there are some strong literature evidences, Agasthiyar gunavakadam is one among them. The inherent proposition of Siddha medical system is based on three humours which are the Vatham, Pitham and Kapham. The potency of the prepared Siddha drugs has high range of therapeutic value.

One of the key factors controlling the absorption of the drug is surface area, decrease in size of the particle directly increases the surface area and thereby aids in increased absorption and bioavailability [10]. Interestingly, in modern science, several researchers have demonstrated enhanced bioavailability of nanoparticles as compared to their bulk form. For example, Ishihara et al. reported higher bioavailability of micronized zinc oxide as compared to standard zinc oxide [11].

It is considered that lead oxide being a major component of the formulation *Singi chenduram* has undergone a major transition from its basic metallic state to another inorganic complex form which renders a potential medicinal property. This transition of lead from one form to another will be achieved only through the prescribed procedure as described by siddhar in the vedic literature. The change of state of the lead was clearly observed from the SEM and TEM analysis of the SC. Spectrum and microscopic nature of the molecular lead oxide present in the SC formulation varies completely from the basic microscopic character of the normal nano sized lead oxide which was well justified on comparing the data's of SEM and TEM of normal lead oxide with lead oxide present in SC formulation [12].
Nanoparticles have important properties that can be used to progress the drug delivery. Where larger particles would have been unequipped from the body, cells adopt these nanoparticles because of their size. Complex drug delivery mechanisms are being developed, together with the capability to get drugs through cell membranes and into cell cytoplasm. Efficacy is important because various diseases depend upon processes within the cell and can only be impeded by the drugs that make their way into the cell [13].

Figure 1: SEM images of *Singi Chenduram*

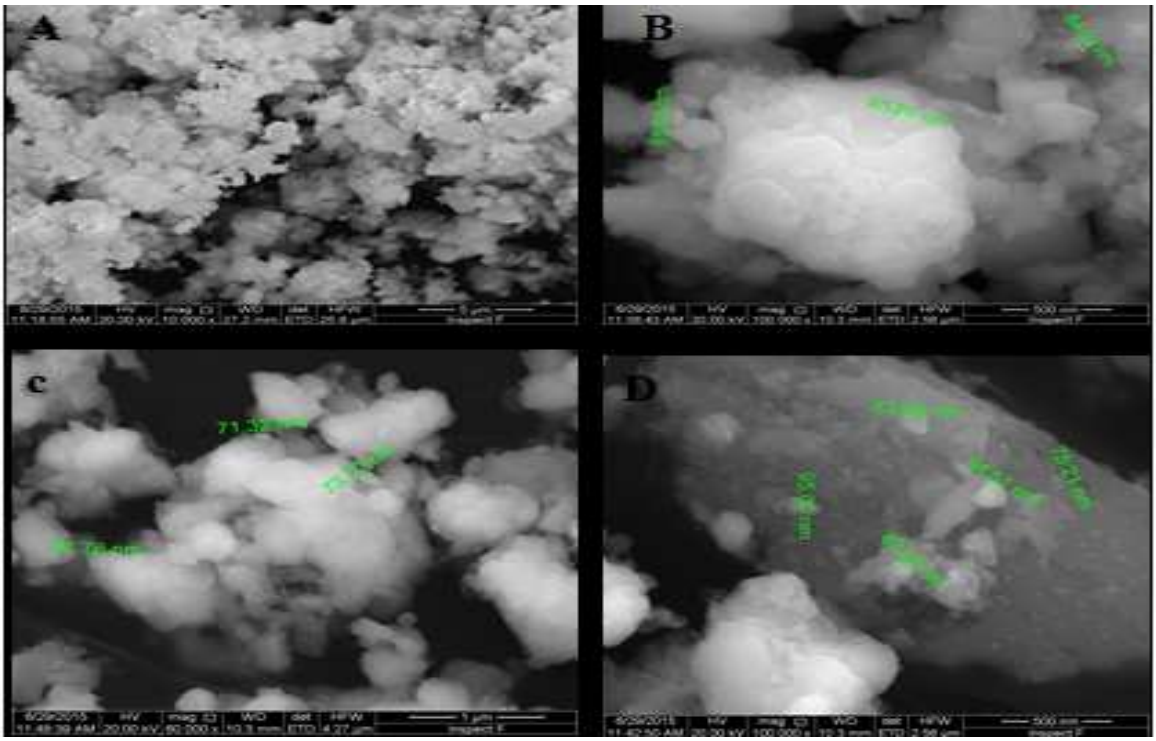
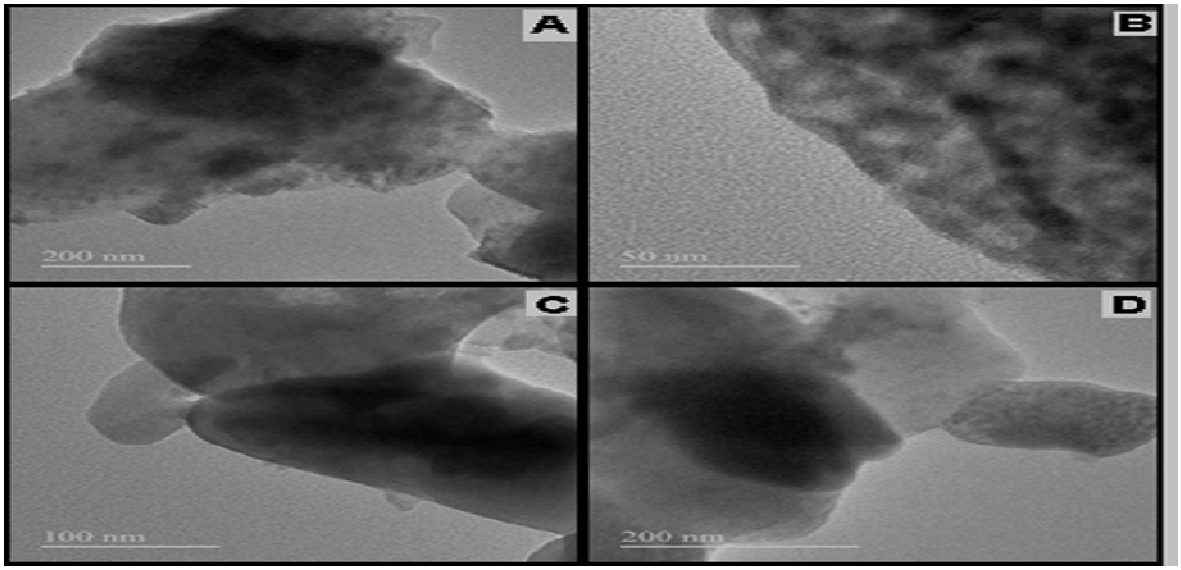


Figure 2: TEM Images of *Singi Chenduram*



CONCLUSION

In conclusion the results obtained from the SEM and TEM analysis of SC reveals some clinically significant result on the nature and size of the nano particle present with in the formulation. It was further concluded that processing of SC formulation though the specified procedures described by ancient siddhars will make the metallic lead oxide to undergo transitional changes from its basic elemental state to inorganic complex from that shows potential therapeutic efficacy. From the literature and experimental datas obtained so far on its nano structure clearly demonstrate that nature of lead present in SC formulation is significantly differ from the normal nano size lead oxide. Nano size particle will easily have access to the biological membrane in particular to mycobacterial species and other bacteria's. Further Anti-mycobacterial effect of SC will be carried out and the relationship between the nano-sized particles with that of its anti-tubercular efficacy will be correlated clinically. Hence in future this evidence based data could provide valuable standard for future researchers willing to pursue their research in *Singi Chenduram*.

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REFERENCES

[1] D.Winston. *The American Extra Pharmacopoea*. **2005**, 1-8.
[2] MY.Wang,JW.Brett, *Acta Pharmacol Sin*., **2002**, 23,1127-1141.
[3] R.Meena,RS. Ramaswamy, *Int J Res Ayurveda Pharm*.,**2015**,6,58-64.
[4] M.Gericke, A.Pinches, *Hydrometallurgy*., **2006**, 83,132–140.
[5] OS.Adriana de, *J Braz Chem Soc*.,**2012**, 13(2): 218-225.
[6] N.Kaushik,MS. Thakkar, S.Snehit,MS.Mhatre, *Nanomedicine: Nanotechnology, Biology and Medicine.*, **2010**,6,257-262.
[7] V.Ranjithkumar,S.Sangeetha,S.Vairam, *Journal of Hazardous Materials*.,**2014**, 273,127–135.
[8] IG.Shibi,G.Shalu,K.Jagannathan,*IJRAP*.,**2012**,3,433-438.
[9] S.Santosh ,B.Kulkarni, *Int Res J Pharm*.,**2013**,4,10-16.
[10] A.Chaudhary,U.Nagaich,N.Gulati,VK.Sharma,*Journal of Advanced Pharmacy Education & Research*.,**2012**, 2,32-67.
[11] K.Ishihara,K.Yamanami ,M.Takano, *Journal of Nutritional Science and Vitaminology*., **2008**,54,54-60.

[12] K.Hasan, K. Mohammad Ali ,H.Saeed,S.Abbas,*Materials Chemistry and Physics* ,**2008**,108,337-344.
[13] X.Zhang, X.Xu, N.Bertrand,*Adv Drug Deliv Rev*.,**2011**,64,1363-1384.