Standardisation of Kushta: Classical and modern approaches

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Abstract
Dosage form, Kushta has long been used and is claimed to be very effective because it is effective in a very small dose and with quick action because of its fast absorbing capability, but serious attempts have not been made to study and standardise it on scientific parameters. In current scenario, it is the need of the hour to standardise this formulation at scientific level. Use of advanced technology in kushta formation needs to be compared with classical method. Approaches for standardisation of kushta mentioned in classical literature should be done. For a standard Kushta, scientific studies should be carried out from the level of organoleptic evaluation, physico-chemical standardization, elemental analysis, microbial evaluation, pharmacokinetics, heavy metal toxicity studies and analytical techniques available for preparation and analysis of Kushta. Recently some studies regarding establishment of quality control of kushtas which have been done by research scholars are discussed in this paper.

Keywords: Standardisation, kushta, Unani, physicochemical, analytical

Introduction
From ancient Greeks to the internet age, the medicinal properties of metals play key roles in human health, but can be toxic if present in excess [1]. However, heavy metals are integral to some simple and compound formulations of Unani medicine and are been used for centuries. Unani formulations containing metals are produced after various purification processes of metals like soaking, quenching, trituration, incineration etc. Hence, metals in the finished products do not possess toxicity. The compounds produced by transmutation of base metal into noble ones along with the use of plant extracts meant to eradicate the toxic effect of metals [2].

Old methods for testing the authenticity of kushtas prepared by classical methods designed on old principles based on limited level of knowledge available at that time are considered obsolete now. Samples of a number of prepared drugs including Kushtas demonstrate remarkable inconsistence in appearance and efficacy probably because of lack of yardstick methods of preparation and standardization of the procedures that ultimately affect the quality of products which, in turn, results in non-reproducibility of efficacy [3].

Traditional calcination techniques or kushtasazi are specialized processes wherein herbal juices are incorporated during preparation of ash. It is claimed that these processes purify and detoxify the metal and make it therapeutically effective and safe [4].

Emphasis has been given on particle size of Kushta in the classical texts. Nano substances (0.1-100nm) can have special physical and chemical properties. Ancient physicians have advised to repeat the process of calcination if the final product is not of required quality (size), which was confirmed only by naked eye examination at that time. It seems true that the ancient were even aware of nanoscience [5].

Unani concepts of trituration and levigation are used to reduce particle size. Particle size determination, however, is not necessary for most of the crude preparations, but it becomes indispensable in dosage form like As a result of different stages of processing techniques like roasting with addition of herbal ices, continuous stirring, wet trituration and puta system of heating, the particle size reduces significantly, which may facilitate absorption and assimilation of the drug into the body system. The particle size in the kushta is 1-2 μ, which could be specified as the criterion for the final product conforming to all the traditional parameters under examination of properly prepared kushta. Nanosizing of particles increases surface area and enhance solubility, increase rate of dissolution, increase oral bioavailability, dose reduction and more rapid onset of action [6].
Though, *Kushta* is a dosage form has long been used and is claimed to be very effective because it is effective in a very small dose and with quick action because of its fast absorbing capability, but serious attempts have not been made to study and standardise it on scientific parameters because of lack of attention of scientific community towards its standardization [7].

**Standardization of Kushta**

WHO says Standardisation is a measurement for ensuring the quality and is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. Standardisation is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect [8].

Nowadays *Kushta* is manufactured in large scales in pharmaceutical industries followed with all advanced technology. Thus until the whole current technical appliances does not meet the classical procedural requirements, the chances of toxicity exist in the form of metal present in above permissible limits. This new approach has given rise to several problems, because the use of new appliances has not been standardized in terms of the detoxification steps carried in classical literature with respect to time mentioned for trituration, quantity of plant extracts used, and the required amount of cow-dung cakes for ignition. Hence, the standardisation is essential with modern analytical techniques of quality control should also be employed along with classical techniques [9].

Standardization of *kushta* is utmost necessary to confirm its identity and to maintain its quality (color/size/palatability), purity, safety, level of toxicity, effectiveness and acceptability of the product by the patients and for Globalization. But the most important challenges faced by these formulations are the lack of complete standardization on all parameters.

For standard *kushta* preparations, scientific analytical studies should be carried out for Physical standardization and elemental analysis, determination of oxidation state of metals and association of these metals with acidic radicals in the finished product, pharmacokinetics of the prominent metallic component of *kushta* using tracer techniques or by metal extraction from tissues, metal accumulation studies in different tissues and organs [10], acute and chronic toxicity studies, expression of heat shock proteins, effect of *kushta* on normal physiological and antioxidant parameters, Therapeutic response of *kushta* on the recommended disease model at cellular and molecular level, role of *kushta* as drug carriers, role of *kushta* in body immune modulation and physiology of gastrointestinal tract. These studies will provide evidence for the safety behind the use of *kushta* and also provide knowledge regarding their mechanism of action [11, 12].

**Raw drug standardisation**

The drug of which *kushta* is to be prepared should be of best quality and it is better to purify it whether it is metal, mineral, animal part or any herb.

**Metal and mineral origin drugs** [11, 12]
- Nature : Crystalline, Amorphous, Opaque, translucent, transparent, aggregate
- Colour : On the freshly broken surface
- Streak : By rubbing the sample in white unglazed porcelain plate
- Cleavage : Tendency of a mineral to split along certain definite plane
- Luster : Minerals reveals its luster at first glance which is produce by the light reflected from the faces of crystals

**Animal origin drugs**
- Collect the sample personally
- It should be healthy animal
- Free from any disease
- It should be freshly acquired
- It should be clean and dried

**Plant origin drugs** [13].
- Identification: Macroscopic examination and Microscopic examination to authenticate herbal ingredients used various process and for carrying out scientific studies with regard to the standardization of raw materials.
- To evolve standards for basic raw materials as well as the raw materials used for *tasfiya* or purification processes after analysis of at least three lots of each raw material procured from different natural sources.
- To evolve standards of purified materials before and after carrying out purification procedure of three lots of materials.
- Quality of plant should be best & newly acquired
- Distilled plant extract is better than any other things.
- Latex, resin, extract should be older than 1 yr.
- The herbs used in preparation should also be of good quality and their *muraqwaq* should be used.

**Process standardisation** [14]

**Classical procedure of Kushta Sazi:** Standard procedure of making of *kushta* stepwise given below, if it is followed properly, it gives a consumable dosage form for human being.

**Collection of raw drugs:** During collection some points are to be remembered like drug should be pure i.e., free from visible and invisible impurities so that hard become brittle and soft because of changes take place in organic and inorganic properties.
- **Soaking:** In Soaking process, Transmutation effects take place, alkaloid binds with metallic compound used in *kushta*, physicochemical changes take place in the presence of air, evaporation of water molecules from surface area making it dry.
- **Trituration:** It helps in particle size reduction and transmutation, facilitates heat transformation (conduction & convection), increases surface area, increases binding property, facilitates uniform mixing and helps in pelletization.
- **Pellitization:** This process involves various steps like drying, preparation of *buta*, again drying, arrangement of pit i.e., *buta* and cow dung cakes, ignition till its self-cooling, taking the material and powdering the material.
- **Gile Hikmat:** This process involves various steps like arranging pellets in *boota*, covered it with another *boota*, sealing it, drying and prevents interference of outside gas and dirt
**Puta system:** During this step, size, type and multiple usage are important factors.

**Aag dena:** Heat causes binding of organic and inorganic constituents, formation of nano particles, hard, heavy, solid converts into brittle, light soft substance. therapeutic value also increases and carbonate, oxides and sulphides are formed.

Some precautions during processing for standard *Kushta* [11, 12, 14]

- *Kushta* should be prepared by an experienced person only.
- Standard parameters should be followed.
- Material used for calcination should be of high quality and free from adulterants
- Weight and measurements should not be compromised
- During trituration, incineration and preservation from dust and air
- Time duration for which trituration, boiling, and incineration mentioned should be strictly adhered.
- Hygienic environment should be maintained while preparation.
- Drug and crucible should be completely dried before incineration.
- Don’t take the *boota* outside from the pit before it is completely cooled.
- Prepared *kushta* should not be exposed to air, moisture and to be stored in air tight glass bottle
- Metals & minerals should be used only after purification as mentioned in text
- If the prepared *kushta* is raw then the process should be repeated from the beginning
- If the intensity of heat used in preparation of *kushta* is not known then it should be experimentally determined starting from giving low intensity heat then gradually increasing the intensity of heat.
- The most important aspect of calcination is heating so it should be done very carefully. While preparing *kushta* from classical method care of wind should be taken, as the place chosen for preparation of *kushta*, should be *mehfuzul hawa* as wind may cause uneven distribution of heat and result in incomplete preparation of *kushta*. Muffle furnace is a better option over classical method preparation of kushta because being a closed chamber furnace gives better temperature control.
- While preparing the *kushta* using classical method one should take care of terms related to the quantum of heat like *gajput*, *kukatput* etc and follow them accordingly. While preparing *kushta* from modern method the important aspect is plotting the thermogram by classical method by using pyrometer and implementation of heat pattern on muffle furnace.
- The weight of the drug mentioned in the formulation should always be followed and should not be changed.
- While preparing *kushta* using classical method it is advised that half of the cow dung cakes are placed first in pit then followed by drug and then it is covered with remaining half of the cow dung cakes. While preparing *kushta* from modern method the sample of drug should be placed in crucible and directly kept in Muffle Furnace. The pellet of triturated dry sample should be placed in center of the crucible.

Unani physicians have explained various methods of *Kushta* preparation. They elucidated several detoxification processes, distinguished between perfect and imperfect *Kushta*, registered ways to turn *Naqts Kushta* to *Kamil Kushta*. All these methods were the classical means of ‘Process and Product Standardization’. Further, the clear indications on the usage of *Kushta* have been instructed. Directions on the types of *Kushta* to recommend and the types of *Kushta* to desist are also mentioned [15].

Before using the metals or metallic compounds, they are always subjected to processes called ‘*tasiyya*’ or ‘*shodhana*’ or purification. This is an idea to get rid of the impurities and their deleterious qualities. If ‘*tasiyya*’ is not performed, their use is said to be injurious to the individual. The physicians believe that the unique and repeated purification processes employed during preparation reduces the toxicity. Further drugs are finely grounded in pestle and mortar with specified juice of known drugs for given time. Then mixture is sealed in an earthen pot using special process and calcinated in closed crucible in pits of different sizes having varying number of cow dung cakes. This provides different intensity of heat. The process is repeated till *kushta* is obtained [16].

The detoxification process claims to purify the metal but very little is known regarding the scientific validation of changes occurring during the procedure and the difference between the *kushta* prepared by detoxifying metal in different medias. Metals and minerals are generally not used directly in the raw form but are converted either into their carbonates or oxides by the process of *Ihraq* or *Taklees*, so as to make them compatible, the product obtained is known as *mukallas* or *kushta* [17].

Some of the medicines, mostly minerals, are subjected to heat under specified conditions and for specific hours, generally they are ground with specified liquids for a particular number of hours or days. After grinding made into *Tikiya* (Discs) and completely dried. These discs are placed in a clay cup and covered with a similar cup so that the mouths of these two cups fit well. Then *Gil-e-Hikmat*, that is, sealing the mouths, is done and dried. The vessels are kept in the centre of cow-dung cakes and placed in a pit specially prepared for it called *Bhatti*. The quantity of cow-dung cakes is generally mentioned in the prescription. Half of the cow-dung is spread over the pits. The cup is placed in the pit and the fire is lighted. Then the other half of the cow-dung is spread over the cups. The pit is prepared in a place where there is no strong breeze. When cooled the cups are removed carefully and the contents collected and ground and preserved in glass stoppered bottles. If the *kushta* is not prepared will during the first process, the above procedure is repeated until the *kushta* has attained perfection [18].

The general procedures for synthesis of *kushta* drugs begin with the process of purification (ghasl-e-adviyah), cleaning (*tasiyya*) and detoxification (*tadbir-e-adviyah*). Further drugs are finely grounded in pestle and mortar with specified juice of known drug for given time. Then mixture is sealed in an earthen pot using special process and calcinated in closed crucible in pits of different sizes having varying number of cow dung cakes. This provides different intensity of heat. The process is repeated till the *kushta* is obtained. The product (*kushta*) has high dissolution rate and ability to get absorbed in the body in a very short period; therefore a small amount of *kushta* induces quick onset of action and high magnitude of effect [19].

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Process standardisation in modern context [20, 21].

- To develop validated Standard Operating Procedure (SOP) for processes involved in preparing the kushta i.e. purification and powdering of kushta
- To evolve standards for “kushtas” including at different stages of heating and powdering of kushta based on at least three lots of powdering of kushta
- To establish validated Standard Operating Procedure (SOP) for heating and powdering of kushta
- Determination of chemical transformation during the above processes.
- To identify modern technology that can be applied to manufacturing of “kushtas.”
- Good quality control
- Multiple batches study at least on three lots of kushta
- Data documentation
- Sampling and testing of in-process material and final product
- Process validation

Process validation means critical process steps that must be controlled within predetermined criteria to eat the product meets its specifications. Critical process steps of kushta may include main drug substance properties, addition drug substance properties (arg, sheer etc.), Quantity and quality of drug substance, size of clay crucible, pit, trituration and drying time, Weight of cow dung cakes, Weight of initial & final drug product and excipients used (making tablet). Typical in-process test for quality attributes for kushta dosage products may be particle size distribution, particle shape and texture, weight gain or lose and hardness of the substances [22].

Final Product Standardisation

Final product standardisation of Kushtas may be done by Specific tests, preliminary tests, physicochemical tests and various analytical techniques.

Classical [23].

- Recently prepared kushta should never be used especially if it is prepared form toxic drugs.
- If needed, kushta should be placed under the moist land for 3-4 days then it should be used.
- The prepared kushta should be stored in airtight bottle not in any pouch made of paper or plastic. Since on size reduction the surface area of a drug is increased.
- The surface area of kushta is more as compared to other dosage forms. So if kushta is not stored properly or due to any other reason is exposed to atmospheric conditions then it will absorb moisture and degradation may occur in such kushta.
- The kushta is generally not used immediately after preparation. It is more effective when used after six months. If urgently needed the kushta should be placed in a bottle. Sealed and buried in moist ground for at least two weeks.

Specific tests

- Gold - Lime Juice (Red)
- Silver - Clove (Black)
- Copper - Curd (Green)
- Hartal - Clotted blood (Dissolved)
- Mica - Morad (Luster)
- Lead - Fire (Red)

General tests or Preliminary tests: These are the basic tests which can be performed without lab and have been performed by ancient physicians in Unani and Ayurvedic system of medicine for a long time [24].
- Floating test: Floating of kushta on surface of water if small quantity of kushta is sprinkled.
- Fineness test: It should be so much fine that on rubbing a small quantity of the sample between the fingers it should enter into the lines lines and creases on the fingers.
- Loss of metallic luster: No metallic luster should be observed when visually examined preferably in presence of sun light.
- Loss of metallic state: No traces of this sample should permanently stick to the silver sheet indicating no alloy formation takes place after heating of a very thin silver sheet to red hot for about 5 min and then allowed to cool. Metallic State can be checked by Maul hayat.
- Wall Stick test: On throwing on the wall, ideal kushta should stick to the wall.
- Fuming test: It shouldnot produce fumes when put on fire.
- Taste lessness: No taste should be observed of kushta.


- Development of physico-chemical & bio assays for standardization of “kushta.”
- To identify modern technology that can be applied to testing of “kushta.”

Physicochemical evaluation

After evaluation for Preliminary properties, following Physicochemical parameters should be tested:

Bulk and tapped density: If 50 gm of kushta is taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume should be noted and the sample then should tapped in Tap density tester until no further reduction in volume is observed [26].

The bulk and tapped densities can be calculated by the formula

\[ \text{Bulk Density} = \frac{\text{Mass}}{\text{Bulk Volume}} \]

\[ \text{Tapped Density} = \frac{\text{Mass}}{\text{Tapped Volume}} \]

Hausner’s ratio: Hausner’s ratio is related to inter particle friction and as such can be used to predict the powder flow properties [19]. The equation for measuring the Hausner’s ratio is

\[ \text{Hausner’s ratio} = \frac{V_o}{V_f} \]

Where \( V_o \) = Unsettled apparent volume and \( V_f \) = final tapped volume.

Compressibility index: It is also known as Carr’s index. It was calculated by following equation:
Carr’s index (%) = \( \frac{[V_o - V_f] \times 100}{V_o} \)

Where \( V_o \) = Unsettled apparent volume and \( V_f \) = final tapped volume.

**Loss of weight on drying:** Two gram of kushta should be taken, spread uniformly and thinly in a shallow petridish. It should be heated at a regulated temperature of 105°C, cooled in a desiccator and weighed. The process should be repeated many times till two consecutive weights were constant. The percent loss in weight can be calculated with respect to initial weight \([27]\).

**Ash value:** \([28]\)

**Total ash:** About 2 gm of the kushta, should be weighed accurately, in a previously ignited and tared silica crucible should be taken. The material should be spread in an even layer and ignited by gradually increasing the heat to 500-600 °C until it is white, indicating the absence of carbon. Ash should be cooled in a desicator and weighed. The content of total ash can be calculated in mg per gm of air-dried powdered drug.

**Acid insoluble ash:** The total ash should be boiled with 25 ml of dilute hydrochloric acid for 5 minutes. Solvent should be filtered through the ash less filter paper and the insoluble matter collected on the filter paper was washed with hot water and ignited to the constant weight. The residue should allowed to cool in a desicator for 30 minutes and weighed without delay. The content of acid-insoluble ash can be calculated in mg per g of air-dried material. The percentage of acid insoluble ash can be calculated with reference to the air dried drug.

**Water soluble ash:** The total ash should be boiled with 25 ml of distilled water for 5 minutes. The insoluble matter should be collected on an ash less filter paper, washed with hot water and ignited. The weight of insoluble ash should be subtracted from the weight of the total ash, giving the weight of the water soluble ash. The percentage of water soluble ash can be calculated with reference to air dried drug.

**Chemical analysis**

1. **pH of 1% solution:** An accurately weighed 1 gm of kushta should be dissolved in accurately measured 100 ml of distilled water and filtered with whatman filter paper. pH can be measured with digital pH meter.

2. **pH of 10% solution:** An accurately weighed 10gm of kushta should be dissolved in accurately measured 100 ml of distilled water and filtered with whatman filter paper. pH can be measured with a digital pH meter.

**Determination of extractive value:** Four gram of kushta should be accurately weighed in a glass stoppered conical flask. 100 ml of water should be added and weighed to obtain the total weight including the flask. It should be shaken well and allowed to stand for 1 hour. A reflux condenser should be attached to the flask and boiled for 1 hour. 25 ml of the filtrate should be transferred to a tarred flat-bottom dish and evaporated to dryness on water-bath then dried at 105 °C for 6 hours. Then it should be cooled in a desicator for 30 minutes and weighed.

**Determination of loss on ignition (LOI):** One gram kushta should be taken in silica crucible and heat to constant weight in electric muffle furnace at 950-1000°C for an hour and then allow to cool. Loss of weight on ignition can be calculated by following equation.

\[ \text{LOI} \% = \frac{[W_3 - W_1] \times 100}{W_3} \]

Where \( W_1 = \), \( W_2 = \) weight of crucible + sample, \( W_3 = \) weight of crucible + sample after ignition.

**Microbiological evaluation:** The contaminants that present serious health hazard are pathogenic bacteria such as Salmonella, Escherichia coli, Staphylococcus aureus, Pseudomonas and other Gram positive and negative strains. Apart from this total aerobic bacterial and fungal count should also be evaluated. According to WHO standards, values of the microbial limits should not exceed 10³ cfu/g for total aerobic bacteria, 10⁵ cfu/g for yeast and moulds, 10 cfu/g for E. coli whereas Salmonella, Staphylococci and Pseudomonas should totally be absent. This should be done to maintain microbiological safety of Kushta \([29, 30]\).

**Heavy metals estimation:** Mercury, Arsenic, Lead, Zinc, Tin, Iron, Sulphur & Copper are mainly utilized for the preparation of Kushta. Out of which Mercury, Arsenic, Lead are heavy metals which are generally toxic in nature. But standard kushta should not contain metals beyond WHO limit for normal permissible concentration of heavy metals \([31]\).

**Analytical Evaluation:** In this modern era, use of technology is becoming more easy and popular. It implies to pharmaceuticals too, as there will be faster production of drugs, standard SOP can be maintained, and results will be reproducible likewise. Hence there is a need in Unani System of Medicine to incorporate new technology in preparation of drugs.

The various modern analytical techniques are described below:

1. **Atomic absorption spectro-photometry (AAS):** When metal/mineral are converted into Kushta (oxides), there are chances of remaining some elemental part in the Kushta even after completion of the process or due to faulty techniques. AAS is a suitable technique for determination of elemental parts present in Kushtas. It is also used in mini laboratories particularly where trace element analysis is required. Pharmaceutical samples may also be analyzed for metal impurities. AAS is a technique by which absorption of light of free atoms is measured. AAS technique is quite expensive because of costly instrument, it can determine about 70 elements (mainly metals) at very low concentrations. AAS is also called a “destructive technique”, because only solutions containing the investigated element can be used. However a very small amount of sample is enough, because of the high sensitivity of the technique. The solvent of the solution is evaporated and all materials present in the sample are vaporised and dissociated to atoms at the very high temperature \([32]\).

2. **X-ray diffraction (XRD):** Atomic planes of a crystal
cause an incident beam of X-rays to interfere with one another as they leave the crystal. The phenomenon is called X-ray diffraction. It is a technique for the qualitative and quantitative analysis of the crystalline materials, in form of powder or solid. Each crystalline solid has its unique characteristic X-ray powder pattern which may be used as a “fingerprint” for its identification [33].

XRD has a good potential for the analysis of nanostructures, because the width and shape of reflections yield information about the sizes of crystallites, microstrain of a lattice, dislocation structures, etc.). G. A. Dorofeev et al studied particle sizes by the X-ray diffraction method are compared by the example of nanoparticles of nickel and iron (3+) oxide (Fe2O3) [34].

X-ray crystallography: X-ray crystallography is a method of determining the arrangement of atoms within a crystal, in which a beam of X-rays strikes a crystal and diffracts into many specific directions. From the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal. From this electron density, the mean positions of the atoms in the crystal can be determined, as well as their chemical bonds, their disorder and various other information [35].

Strengths of XRD

- It can determine the orientation of a single crystal or grain.
- It can measure the average spacings between layers or rows of atoms.
- It can find the crystal structure of an unknown material.
- It measures the size, shape and internal stress of small crystalline regions.
- XRD is a non-destructive, fast technique.
- It has easy sample preparation.
- Powerful and rapid (<20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straightforward

Limitations of XRD

- Homogeneous and single phase material is best for identification of an unknown
- Must have access to a standard reference file of inorganic compounds (d-spacings)
- Requires tenths of a gram of material which must be ground into a powder

3. ESCA: (Electron Spectroscopy for Chemical Analysis) or XPS (X-Ray Photoelectron Spectroscopy): It is an efficient surface analysis technique that provides elemental and binding energy information about a material's surfaces and interfaces. Basically it is used to determine electronic nature and oxidation state of metal. ESCA analysis can detect all elements from lithium to uranium. Electronic Nature and oxidation state of metal [36]. This technique faces drawbacks due to interactions with the substrate and sample charging effects. The structure of the Si/SiO2 interface was probed without any substrate interaction or charging effects for silicon nano crystals previously oxidized in ambient air. Complete characterization of the surface was obtained. The Si 2p core level spectrum reveals a non-abrupt interface. The ability of X-ray photoelectron spectroscopy (XPS) to provide information on the local bonding environment of a given species makes it an essential tool for understanding the surface chemistry of materials [37], [38].

4. Differential Scanning Calorimetry (DSC): Differential Scanning Calorimetry (DSC) is first developed in the early 1960s. It is a thermal analysis procedure that measures the rate of heat flow as a sample undergoes phase transitions. DSC helps identify exactly how much heat is needed to transform a sample from one state (solid) to another (liquid). Differential scanning calorimetry can be used to analyze the rate of both endothermic and exothermic phase transitions by measuring the rate of change in the test sample against that of a known reference material. Technical improvements over time make DSC a very relevant tool for investigating the thermodynamic properties of various pharmaceutical products, such as, biopolymers, proteins, peptides, and lipid carriers [39].

5. Scanning Electron Microscopy (SEM): To obtain information about a sample's surface topography and composition SEM technique is used. Digital image resolution as low as 15 nm. Magnification for all imaging is calibrated to a traceable standard. Image analysis for coating thicknesses, grain size determinations and particle sizing can be applied to the saved images. Qualitative elemental analysis, standard less quantitative analysis, x-ray line scans and mapping can be performed on both of the SEM systems [39].

6. Transmission Electron Microscopy (TEM): It is a Single particle technique. Compositional and crystallographic information can also be obtained. Typical range is 5nm to 500μm. It is used to determine Particle size and distribution. The transmission electron microscopy (TEM), Brunauer–Emmett–Teller (BET), and X ray diffraction (XRD) methods are most often used to measure the sizes of particles and crystallites in polycrystals in nanoscale. TEM is a direct method of observation; it is most preferable, especially when studying the fine structure of crystallites. However, it has some peculiarities and limita tions. The method is laborious for sample preparation; e.g., very small particles can be transparent for electrons and not exhibit a contrast. In addition, there are serious problems relevant to the observation of fine nanostructures in rather large polycrystalline samples [40].

7. Atomic force microscopy (AFM): AFM has several advantages over SEM/TEM for characterizing nanoparticles. Images from an AFM represent data in three dimensions, so that it is possible to measure the height of the nanoparticles quantitatively. With an SEM/TEM, the images measured are only two dimensional. AFM scans more slowly than an SEM. However, a complete measurement session that includes sample preparation, acquiring an image, and then analyzing the image takes much less time with an AFM. In fact, typically it takes about 1/4 of the time to
get data from an AFM than with an SEM/TEM. An AFM is a very cost effective microscope for nanoscale imaging. In general, an AFM with the comparable resolution to a SEM/TEM costs much less than the SEM/TEM. Further, the AFM requires substantially less laboratory space than an SEM/TEM; only a desk or possibly vibration table is required for an AFM. And finally, the AFM is much simpler to operate than the SEM/TEM, so the AFM does not require a specially trained operator [41].

8. Thermo gravimetric analysis (TGA): It is a thermal analysis procedure that measures weight loss as a function of temperature in a controlled environment. The temperature points at which active components volatilize are plotted, creating a graph that quantifies the composition. This type of analysis can be useful in determining differences in polymers, especially in the amounts of inorganic filler used within the polymer [42].

9. Flame atomic absorption spectroscopy (FAAS): FAAS is the simple, fast and reliable oldest of these techniques and relies upon the electrochemical properties of metals that allow them to absorb energy from light of specific wavelengths. The relationship between the amount of light absorbed and the concentration of analytes present in known standards can be used to determine sample concentrations by measuring the amount of light that they absorb. A method has been developed for the separation/preconcentration of trace amounts of silver ions using 2-mercaptobenzothiazole/sodium dodecyl sulfate immobilized on alumina-coated magnetite nanoparticles and their determination by flame atomic absorption spectrometry [43, 44].

10. Graphite furnace atomic absorbance spectroscopy (GFAAS): GFAAS is similar to FAAS, but uses a different sampling system. GFAAS uses an improved sampling device that atomizes the entire sample and retains it in the light path for an extended period of time. This is done by replacing the flame used in FAAS with an electrically heated graphite tube. These changes significantly improve the detection limits of the technique [45].

11. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES): ICP-AES uses argon inductively coupled plasma maintained by the interaction of a radio frequency field and ionized argon gas to excite atoms to unstable energy configurations. The wavelengths of the energy released are specific to the elements in the sample, and the intensity of the emission is a function of the concentration of atoms that are affected. ICP temperatures reach as high as 10,000 degrees Kelvin with samples experiencing temperatures between 5,500 and 8,000 degrees Kelvin. (Aulton EM,2009) Measurements of Cu, Mn, Cr and Ni by inductively coupled plasma atomic emission spectrometry (ICP-AES). ICP-AES done for simultaneous determination of trace elements in biological sample and water [46].

12. Inductively coupled plasma-mass spectroscopy (ICP-MS): ICP-MS retains the sample introduction system used in ICP-AES. The MS separates the ions introduced from the ICP according to their mass-to-charge ratio. Ions of the selected mass-to-charge ratio e directed to the detector, which records the ions present. This provides identification and quantification of the elements of interest. Typically a quadrupole mass analyzer spectrometer is used due to its ease of use and speed. However, other mass analyzer systems such as ion-trap, sector field, and time of flight can be used [47].

13. X-Ray Fluorescence Spectrometry (XRF): It is seeing some use as a screening tool due to the availability of hand-held field instruments. XRF employs x-rays to ionized elements and records the characteristic emissions of atoms as they return to more stable energy states. It is fast, relatively inexpensive, requires minimal sample preparation, can identify many elements at once, but is only moderately sensitive [29].

14. Energy-dispersive X-ray spectroscopy (EDS, EDX, or XEDS), sometimes called energy dispersive X-ray analysis (EDXA) or energy dispersive X-ray microanalysis (EDXMA), is an analytical technique used for the elemental analysis or chemical characterization of a sample. It is a single particle technique. Compositional information can be obtained with EDS. It is used to determine chemical nature, size and morphology of particles [29].

15. Dynamic light scattering: It is also known as photon correlation spectroscopy or quasi-elastic light scattering. It is a technique used to determine the size distribution profile of small particles in suspension or polymers in solution. DLS is used to characterize size of various particles including proteins, polymers, micelles, carbohydrates, and nanoparticles. This technique commonly used in chemical and pharmaceutical industries. It determines the size distribution profile of small particles in suspension or polymers in solution. It relies on Brownian motion of particles in a liquid medium to determine particle size. Typical range of size of particles is 50nm to 1μm.

16. FTIR analysis (Fourier Transform Infrared Spectroscopy) is a technique to identify organic (and in some cases inorganic) materials. Presence of toxic elements elements beyond the permissible limit is not acceptable. Fourier Transform Infra-red Spectroscopy validates the authenticity and purity of a sample. As for as this technique is related to Kushtas it will not only identify the sample, but also give an idea about intermediate products and functional groups if compared with reference sample i.e., FTIR Analysis also used to: Identify unknown materials, Identify and/or quantify surface contamination on a material, Identify additives in a polymer, when they can be chemically removed, e.g. solvent extraction. FTIR analysis measures the range of wavelengths in the infrared region that are absorbed by a material. In a research Silver-Poly (Methyl methacrylate) Nanocomposites have been analysed by FTIR technique. The FTIR spectra confirm the complexation and interaction among the compounds. The interaction persuades the structural interphase alterations in the Ag/PMMA nanocomposites [48].

Recent studies in Kushtasazi
The tests and observations used to monitor quality of kushta described in the Unani text are highly subjective. Recently some studies have been carried out regarding establishment of quality control of some kushtas have been done by research scholars and they used all the advance techniques

Conclusion
Kushta dosage form is the oldest form of nanotechnology. In spite of the fact that Kushta have been used since centuries without any obvious side effects and is well-established in Unani Medicine, very little scientific research have been carried out in respect of standardisation. In spite of wide scope for incorporation of modern tools and techniques for standardization of this dosage form, many queries such as temperature standardization, quantum of heat required for the process, reasons behind mixing herbs with metals before preparation and many other unsolved questions are remained which may be answered by modern scientific researches. In spite of being effective mainly in chronic diseases, Kushta dosage form has not been introduced globally. It becomes imperative to study all the aspect of Kushta and explore the hidden mysteries about this dosage form so as WHO globalize it like some other dosage forms. SOP and SMP should be strictly followed as described in Unani literature followed by modern techniques. Physicochemical characterization of Kushta by using modern technologies is also an essential prerequisite. It is also a considerable and wide field for academics and research interest. Use of advanced technology in kushta formation needs to be compared with classical method as the soul of kushta lies in its method of preparation. If procedure of kushta is correct, certainly the functions will be marvellous. So, methodology should be improved by scientific validation.

Conflict Of Interest
The authors have no conflicting financial interests.

References
24. Kabiruddin M, Bayaze Kabeer. Part-III. Hyderabad:
and their determination by inductively coupled plasma atomic emission spectrometry in water. Analytica Chimica Acta 2001; 440:207-213. ELSEVIER
56. Tariq M, Chaudhary SS, Zaman R, Rahman K, Imtiyaz S. Formulation and physicochemical evaluation of a