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Study on physical properties of *Ayabirungaraja Karpam* engaged by Traditional and Modern technique

Research Article

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Abstract

The physical characterization is an authentic proof for the metal and mineral drug formulations used in traditional system of medicine. Now a day quality assurance is a thrust area for the evaluation of traditionally used medicinal plants and herbal formulations. In this research work, author aims to elicit traditional and modern physical characterization of Siddha formulation *Ayabirungaraja karpam*(ABK). It was prepared as per Siddha reputed text classical method and subjected to physical characterization traditionally such as consistency, lightness, texture, particle size, colour, odour, tactile sensation, irreversible nature and modern parameters like pH value, Loss on drying, Total ash, acid insoluble ash, water soluble ash, Bulk density, Tapped density, Zeta size, Zeta potential and BET analysis for surface area. The results reveal as per traditional trait, ABK is non-adhesive freeness, fineness and lightness which enhance absorption and assimilation of particle in body without producing any irritation to the mucous membrane of gastrointestinal tract. Modern parameter indicate the presence of organic moieties as well as mineral, acidic medium which was not favour for microbial growth , cohesive, low porosity and compaction of drug powder was confirmed. Particle size matches well with colloidal size which attached to human intestine and thus provide large surface area, thereby increase absorption of macro, micronutrients and drug. Therefore, it is concluded the drug is user friendly for human oral administration.

Key Words: Characterization, Absorption, Mucous membrane, Traditional formulation, Efficacy, Surface area.

Introduction

The quality control aspect has been covered by careful observation of skilful traditional medical practioner. The current researchers believe that the ancient scholars of traditional medicine do not have any idea about drug standardization but siddhars' wrote in their 'ola' manuscript about infinitesimal physical and chemical quality control parameters to regulate the quality, efficacy and safety of the product in their period. (1) In modern concept, it requires necessary changes in their approach by way of quality control in terms of development of modern methodologies. Thus, today quality assurance plays vital role for the evaluation of traditionally used medicinal plants and herbal formulations. Manufacturers who are doing some testing of their formulation have fixed their own parameter, most of them are only preliminary in nature. Integrated genuine efforts from scientific workers of different disciplines are required for this purpose. (2)

Humans have been used natural products from the source of plants, metal, mineral and animal products to get relieve from the illnesses since 7th century AD.(3) The use of natural products as medicines faced lot of challenge to early humans because they may have certain defects, even though they are valuable natural resource of human expertness. Invent new drugs depend only on modern technology appears to be reaching some limitation because pharmaceutical industry has adopted chemical based synthetic combination since 1980s. This direction has not resulted in the successful drug productivity. Some reputed pharmaceutical companies were facing great challenges to develop new products, then increasing attention has paid on natural products in the search for new herbal drugs in pragmatic way of approach along with high capacity sophisticated equipments.(4)

The physical characterization is an authentic proof for the metal and mineral drug formulations used in traditional system of medicine. It not only eliminates toxicity of metals but also make efficacious medicinal product for human beings and beneficial for the treatment of many diseases. This enhances the acceptability of traditional system of medicine all around the globe. (5) Various method of physicochemical analysis outcome revealed that the traditional formulations possess raw metals for their preparation lose their metallic characteristics and turn into mineral complex after processing. In their raw form, the metals like mercury, copper, iron, sulphur and lead etc. would be highly toxic. However according to the ancient text of the traditional manufacturing process emphasize long duration of pharmaceutical processing whereby the ingredient are

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grinding and heating cycles repeated several times with each cycle lasting 4-7 days and fired in furnaces at temperatures up to 6000 °C are believed to remove the toxicity and impart remarkable therapeutic value to the drug.(6)

During the various steps of pharmaceutical processing, the triturating process will make easier the ingredient materials mixed uniformly and divided into fine particles. Surface area of material exposed and expanded that facilitates reaction during heating process where materials become soft, smooth and sticky which facilitates better binding, thus enhances instant assimilation and absorption of metallic and mineral preparation. The basic material when treated frequently with plant extractives and heated on fire, the plant extractives are converted into ash or solid organic or inorganic forms depend upon the intensity of heat applied, compounds are reduced and converted into another compounds, elemental metals gradually reduced and converted into compounds.(7) Standardization of metal mineral formulation is utmost necessary to confirm its identity and to determine its quality, purity safety, effectiveness and acceptability of the product. But the most important challenges faced by these formulations are the lack of complete standardization by physiochemical parameters.(8) Standardization 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 and detection of its nature of adulteration.

As per the Siddha metallurgy, *Ayabirungaraja karpam* is a siddha product of herbo-metallic drug and contains both metallic and herbal ingredients. Since it is classified under *Kayakarpam* it is more stable over a longer period of time, easy to store and sustainable availability like Bhasma and Chenduram. Now a day, there is an urgent need for the practitioners of traditional systems to standardize the synthesis procedure for scientific analysis of metal-based drugs. Based on the principle and classical method for drug preparation in Siddha medicine, the drug ABK contain iron ore *(Ayam)*, iron cast *(Mandooraam)*, were underwent detoxification process for elimination of harmful matters. Impregnation and solar heating with the herbal juices such as *Wedelia chinensis* and *citrus limon*. (9) The conversion of iron present in the free form gets converted in to iron oxides. Thereby the researcher decided explores the physical properties of ABK by traditional technique and modern scientific techniques to gather scientific evidence to corroborate the authenticity.

Materials and Methods

Procurement and Authentication

The main ingredients raw ore iron (*Ayam)*and raw cast iron (*mandooram*) were purchased from Trichy local market and authenticated by Dr.KKadirvelu, retired Professor of Geology, V.O.Chidambaram College, Tuticorin. *Wedelia chinensis*(Osbeck) Merr (Voucher specimen No CARISM 109) was collected from local herbal garden, Thanjavur and *Citrus limon*L(Voucher specimen No CARISM 110) fruit was purchased from local market ,Thanjavur. Both herbal raw material were authenticated by Dr Ravichandran, Asst.Professor in Botany, CARISM, Sastra University, Thanjavur.

Purification of raw materials

Iron(*ayam*) 200g was heated in the pan until become red hot and subsequently quenched in 300mL 6 months old *Anna khadi*, then washed out by water. The process was repeated three times for heating, quenching and washing while every time fresh *anna khadi* and water were used. The same purification steps were carried out with 300mL,Sesame oil, 300mL Cow urine and 300mL *kollu kudineer* respectively. Altogether twelve times putout in the four types of different liquids to obtain purified form of *Ayathool*. The next ingredient of *Manduram* 300g was weighed which was grounded well and put in a pan and heated up to red hot, then added 4 times weight of Tamarind leaves , 8 times of water and boiled for 3h. Once cooled *mandura* powder was rubbed with boiled and macerated tamarind leaves while washing. Then allowed to dry and residues were removed from the powder. The next step of purification process was heating this *mandura* powder with 8 times weight of cow urine, and then washed out to obtain final purified *mandurm.*

Preparation of *Ayabirungarajakarpam*

Purified *Ayathool* and *Manduram* were mixed well in 2:3 ratio and they were drenched in *Wedelia chinensis* juice and lime juice separately, the mixture was kept under sunlight heat (*Sooryapudam*) for drying under solar heat. Again, the drying process was repeated by adding only *Wedelia chinensis* juice until become waxy consistency. During the drying, the product was regularly mixed with spatula, and then it was transferred to herbal cup (thonnai) for complete drying under solar heat. Then the grey color fine powder was obtained after grind this final product.

Traditional Evaluation Parameters

In ancient traditional method of standardization, physicochemical methods are often used to determine the consistency, texture, lightless (10), particle size, colour, irreversible nature(11), tactile sensation, taste and odour. (12)

Consistency

This method is performed by tactile sensation to determine adhesiveness or freeness of ABK drug by rubbing a pinch of powder between fingers to ensure the freeness of particle.

Lightness (*Ilahu*)

ABK powder 5mg and a clear glass beaker with 100mL water were taken for testing floating ability by sprinkling the drug powder gradually on motion less water surface from a short distance.

Particle size and Fineness

ABK powder 5mg was placed between thumb and index fingers were thoroughly rubbed and filled with furrows of finger tips.

Tactile sensation

To elicit this test touch the ABK powder by finger tip, the proper finished herbomineral drug attained this quality.

Colour (*Varnam*)

ABK powder 5g was taken in a clear glass beaker and tested for colour by observing against a white opaque background under solar light and should be confirmed with two individuals.

Taste *(Suwai)*

ABK powder 5gwas soaked in the pure and clean cotton bud which was placed in the different six taste areas of tongue.

Odour (*Manam*)

ABK powder 5g was put in the beaker and tested for its odour by blowing the air above the beaker.

Texture (*Thanmai*)

ABK powder 5g was observed under bright sunlight for the typical 'lustre' character (deoxidized state).

Irreversible (*Meelukai*)

This experiment was carried out by mixing ABK with equal quantity of seeds of *Abrus precatorious*, ghee, borax, honey and Jaggery which all put together in the pot made of earth and sealed. Uniform flame heat system was applied for half an hour and allowed for self cooling.

Modern scientific tool

The scientific instruments and methods are used to find out the physical properties of ABK.

Estimation of pH Value

Measurement of pH is a useful test to know the acidity or alkalinity of test drug ABK aqueous solution. The temperature was set at 25°±2°C. Potentio metrical method was used to determine the pH value of the solution with the aid of a glass electrode, a reference electrode and a pH meter of digital type. Apparatus was calibrated using buffer solutions, adjusted the meter to read the appropriate pH value corresponding to the temperature of the solution. The electrode was immersed in the solution being examined and measures the pH at the same temperature as for the standard solutions. At the end of a set of measurements, pH of the solution was recorded. If the difference between the reading and the original value is greater than 0.05, the set of measurements were repeated. (13)

Loss on drying (LOD)

Determination of loss of weight in % w/w was measured using LOD resulting from the removal of water and volatile matter. Weighed glass stopper petri dish which has been dried with the same manner to be employed in the determination. Two gram of ABK sample was transferred to the dish which covers it and accurately weighed. The sample was distributed evenly. The stopper was removed and placed the loaded dish in the drying chamber as directed in the monograph. The sample was dried to constant weight or till two consecutive weights remains within ± 0.5 mg. After drying is completed, cool the sample in desiccators before weighing and the weight was noted. (13)

Loss on drying (0/0 w/w) = Loss in weight (g) ×100/ mass of the sample (g)

Total ash estimation

To determine the percentage of ash of the ABK sample, 2g of the air-dried material was weighed accurately in a previously ignited and tarred silica crucible. The sample was ignited in a muffle furnace at a temperature not exceeding 450°C until free from carbon. Cooled in a desiccators and weighed. The charred mass was enervated with hot water, the remnant was collected on an ash less filter paper, the remnant and filter paper was incinerated until the ash was white. Filtrate was dry out and ignited at a temperature not exceeding 450°C.The % of total ash was calculated as follows(13)

Percentage of total ash (0/0 w/w) =Mass of ash (g) ×100/Mass of sample (g)

Acid insoluble ash

To determine the percentage of acid insoluble ash of the ABK, the ash was added with 25mL of 2M Hydrochloric acid and boiled for 5 minutes. Insoluble matter was collected on an ash less filter paper and and was washed with hot water until free from acid. Cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated as follows. (14)

Percentage of acid insoluble ash (0/0 w/w) =Mass of acid insoluble matter (g) ×100/mass of the sample (g)

Water soluble ash

To determine the percentage of water soluble ash of the ABK sample,the crucible containing the total ash, added 25mL water and boiled for 5min and collected the Insoluble matter in an ash less filter paper and washed with hot water, then ignited for 15min at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and the difference of the weight represents the water soluble ash. (14)

Percentage of water soluble ash (0/0 w/w) =Mass of the insoluble matter (g)×100/mass of the water soluble ash

Bulk density

Bulk density is defined as the dry weight of powder sample. the high bulk density is an indicator low porosity and compaction of sample powder. It is the ratio between given mass of a powder and its bulk volume. ABK 100g powder samples was passed through sieve with apertures greater than or equal to 1mm, if necessary, to break up agglomerates that may have formed during storage. This must be done gently to avoid changing the nature of the material in to a dry graduated cylinder of 250mL. Approximately 100g of the test sample was weighed and gently introduce without compacting level of the powder without

compacting and was read the unsettled apparent volume (Vo) to the nearest graduated unit. Measure the combined mass of the sample ad graduated cylinder to nearest 0.1g and value was recorded. (14)The bulk density of a material is the ratio of the mass to the volume of an untapped powder sample

$$\text{Bulk density} = \frac{\text{Mass of a powder}}{\text{Bulk volume of the powder}}$$

Tapped density

Tapped density is an increased bulk density attained after mechanically tapping a container which contains the powder sample. The tapped density describes a bulk density of a powder after consolidation or compression (tapping), the container of powder measured number of times, usually from a predetermined height. Tapped density of ABK was measured by using tapping graduated glass measuring cylinder manually. The raising and lowering of the cylinder by hand was done either without reference to the height transverse arbitrary acceleration in both up word and downward directions. The repeated tap was given by striking its base down on to a hard surface. Once observing the initial powder volume and weight, the measuring cylinder is gently tapped, and then volume or weight reading was taken until further volume or weight changed. The change in tapped power volume relates to flow properties of powders. It can be determined by compressibility which is computed from powder density using the following equation. (15)

$$\% \text{ of Compressibility} – 100(Vo\text{-}Vf)/Vf$$

Vo –Unsettled apparent volume; Vf –Final tapped volume

Flow property

Flow property is usually assessed by determining angle of repose of the powders. Inter particulate friction or resistance to movement between particles depends on angle of repose. It is defined as the constant three dimensional angle measured relatively to the horizontal base, assumed by a cone-like pile of material formed when the powder is passed through a funnel-like container. The trial drug ABK powder was allowed to fall over a paper on a horizontal surface through a funnel kept at a certain height. The powder was then poured in to the funnel and then the funnel was gradually introverted without any shaking movements allowing the powder to make a heap on the horizontal surface. The height of the heap is measured and then the circumference of the base of the heap was drawn on the paper with the help of pencil. [16]

The angle of repose was given by $\tan\theta = h/r$

The height of the heap(h) – (that is the distance between the horizontal surface and the lower tip of the panel)

Radius of the base of the pyramid. (r)

Zeta size (Particle size)

The particle size distribution of ABK sample was obtained by dynamic light scattering using zetasizer Nano ZS (Malvern Instruments, UK).In the black scattering mode(angle detection 173^0,measuring position 1mm from the wall of cuvette), the instrument identify the time increase of the power of light scattered by moving particles in the samples after irradiation with the He-Ne laser beam (5mw,633nm).The data obtained by autocorrelation analysis and were subsequently evaluated using the methods of statistical data analysis(both Cumulant analysis and CONTIN algorithm) to obtain the particle size distribution. The measurement was taken at laboratory temperature. (17)

Zeta potential

The zeta potential of the ABK samples was evaluated by the method of electrophoretic light scattering using Zetasizer ZS instrument (Malvern instruments UK).The zetasizer identify the Doppler shift between the laser beam (5mW, 633nm) passing through the cuvette with the ABK sample and the reference beam passing outside the cuvette. The data were evaluated using phase analysis light scattering A Zeta dip cell was used to introduce an electric field in to the sample (applied voltage 4.96±0.05). The measurement was taken at laboratory temperature. (18)

Brunauer-Emmett-Teller(BET)

BET was warmed up for at least 30 min before starting the experiment. For an analysis, the ABK is filled in an instrument specific glass holder and it was weighed three times on a microbalance. Afterwards the sample of ABK dried powder was put in the instrument being evaluated, heated up to specific time and temperature. The sample was cooled down and weighted again to ascertain the possible mass loses. The sample holder was placed in the BET measurement unit and the analysis was commenced by cooling down the sample, followed by nitrogen injection under various pressures to determine the Nitrogen displacement for the surface area calculation.(19)

Results and Discussion

The consistency test results shows the ABK is non-adhesive which enhances absorption and assimilation of particle in body without producing any irritation to the mucous membrane of gastrointestinal tract(GIT). Therefore the freeness, fineness and lightness are the ancient physical standard parameters which assumed that do not cause any irritation to mucous membrane when it comes in contact. The lightness (*Illagu*) test results show the trial drug ABK powder floated and formed a layer in the water surface due to low surface tension. When dried rice grain was placed on the floating drug layer, it does not disturb the layer which confirmed the lightness and excellent finishing stage of ABK.

The fineness test indicated the furrows like “loops” or “whorl pattern on finger. It is assumed that the ABK powder has minute particle size and thus, facilitates the easy absorption and assimilation of drug. The tactile sensation expressed that the ABK powder will not irritate the mucous membrane of the GIT.ABK powder was confirmed as lustreless in texture because after unique process of herbomineral preparation. The colour test of ABK results showed greenish black colour powder. Each and every drug have the specific colour according to their raw material colour and

chemical nature of the drug, thus iron metals treated with herbal extract give this specific colour. The taste test of ABK showed as astringent taste in all six areas of taste buds in the tongue, the transformation of metallic particles by unique process with herbal extracts gave astringent taste for ABK powder. The typical lime odour was obtained in the final product. In ability to regain of metallic test showed, the metal ingredient of ABK did not revert to the original state where as no presence of free metal noticed which indicates the proper processing of ABK.

Figure:1 Floating test of ABK

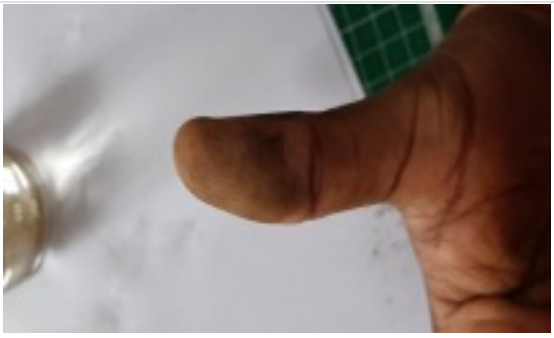

Figure: 2 Furrows filled with drug powder

Figure: 3 Finger printing of drug powder

Loss on drying (LOD) test represent the moisture drying off from the drug, the moisture content ranging from 10-20% is ideal range for minimal bacterial and fungal growth. The observed moisture content 6.923% w/w±0.0183 of the ABK drug powder is highly significant and within the standard limit of pharmacopeia standard of *Ayurveda* which prevents decomposition of drugs either from chemical change or microbial contamination. Thus, low moisture content of ABK prevent reduction of efficacy and degeneration this indicate the long shelf life of trial drug. (20)Therefore, This was evidenced by siddhar's quotation since the shelf life of karpam has been dated up to 75 years.(21) Total ash value is the criteria to judge the identity and purity of the drug and it was found to be54.69% w/w±0.0370 which indicate significant amount of organic matter as well as the mineral were available. The maximum percentage of ash value of ABK indicates the presence of metal and mineral compounds in the drug. (22)

The drug possess a moderate acid soluble ash value of 33.56% w/w±0.0025 indicating that the preparation does not contain any silicaceous matter.(23) The water insoluble ash of ABK was found to be2.040%w/w ± 0.1609 which indicate the fair solubility in water. The pH value at 25^0C was found to be 4.12 which indicate the acidity of the drug. pH value influences the quality of medium and control many chemical and microbial reaction.(24) Low pH value indicate the drug is in acidic medium which is not favour for microbial contamination. (25) The higher value of bulk density and tap density decide the bulkiness of the powder and poor flow property. The observed result of bulk density of ABK was found to be 0.837gm/ml and tapped density is 1.116gm/ml. Bulk density is defined as the dry weight of powder sample. . Bulk density is obtained by adding known mass of powder to a graduated cylinder. The cylinder containing the powder is tapped by repeatedly striking its base down on to a hard surface. The changed in tapped power volume related to flow properties of powders. The high bulk density is an indicator low porosity and compaction of sample powder.

The bulk density plays the role considering the size of high dose capsule product, homogeneity of low dose formulation where vast differences may occur between drug and excipient densities, knowing the dose and formulation density to determine the appropriate size for a capsule formation. The inter-particulate interaction that influence the bulking properties of a powder and also the interaction that interfere with powder flow. It is therefore possible to gain information about the relative information of this interaction of ABK sample by comparing the bulk and tapped density which is used to index the ability of powder to flow. In a free flowing powder these interaction are less significant and the tapped or bulk densities will be closer in value .The poorly flowing materials which are greater inter particular interaction and a greater difference between bulk and tapped densities will be noticed. The observed results show good character of the drug *Ayabirungaraja karpam.*

The flow property of ABK was found to be 58.29^0 the comparison with the normal range of angle of repose, the result of flow behaviour of ABK is very cohesive. The angle of repose has been used in various branches of science including pharmaceuticals to characterize the flow property of solids. Angle of repose is characteristically related to inter particulate friction of

particles.(26)The angle of repose of the granular matter is the steepest angle of descent or dip relation to the horizontal plane to which the material can be piled without slumping.(27)The angle repose can range from 0-90^0 and the Reference value of angle of repose in degree is given as follows. (28) (29)

Table: 1 Reference value of angle of repose in degree

Angle of repose^0	Flow behaviour
25-30	Very free flow
30-38	Free flow
38-45	Fair flow
45-55	Cohesive
< 55	Very Cohesive

Table: 2 Zeta size dispersion

			Size d.nm	Intensity%	Std.Dev. d.nm
Z. average (d.nm)	3455	Peak 1	4605	85.5	844.7
PdI	0.789	Peak II	337.5	14.5	114.2
Intercept ion	0.102	Peak III	0.000	0.00	0.000

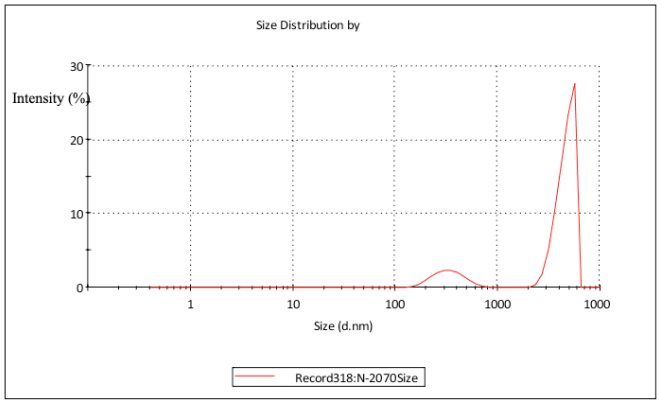

Figure: 4 Zeta size dispersion on ABK

Zeta size dispersion on ABK nano particles at intensity and size 3455 d.nm is shown in figure 4. The physicochemical properties of nano particle such as particle size and zeta potential are important for understanding the interaction of nano particles with biological system.(30) The recent past research study of FE-SEM revealed that the roughness morphology seen on the surface of ABK drug may be due to the aggregation of nano particles during various processes on drug preparation. (31) Particle size 1-2 µ may facilitate absorption and assimilation of the drug into the human body system.(32) ABK particle size is 3455d.nm and the particle was stabilized adding lime juice contain citrate in the preparation of ABK, have the ability to migrate into cells and body compartments is due to its unique small size. The particle size found by zeta sizer is higher than the particle size seen in microscopic studies with SEM. This is due to the aggregate of the nano structural particle. Organic molecule present in the ABK interacted with water on zeta size analyser. Therefore the size is found to be micrometric level in zeta sizer. *Ficus benhalensis* leaf contain high level of water soluble antioxidant, polyphenols (flavanoid) (33) Phenolic compounds possess hydroxyl and ketonic group which are able to bind metal and show chelating activity. Protein and enzymes present in the *F.benhalensis* leaf extract facilitate the formation of metal nano particle. Therefore using *Ficus benhalesis* herbal cup *(thonnai)* in the preparation is a rational to reduce the particle size and thus facilitate for high bioavailability by forming nano particles. The size of the particle is reduced to nano particle which passes through sieve no 44 indicating the fineness of the drug which facilitates the easy absorption and assimilation. (34) The particle size matches well with colloidal size which attached to human intestine and thus provide large surface area, thereby increase absorption of macro, micronutrients and drug. The presence of organic matter on the surface of the drug suggest it act as coating material in the surface of the metallic compound. Therefore, the ABK pharmacologically act as well absorbent and. adsorbent. The nano particle size of ABK form association with organic molecules and enhance the biocompatibility of the drug.

Table: 3 The results of Zeta potential

			Mean (mV)	Area %	St.Dev (mV)
Zeta potential (mV)	-13.6	Peak I	-15.3	96.6	6.19
Zeta Dev. (mV)	8.62	Peak II	15.6	3.8	2.95
Conductivity (Ms/cm)	0.0950	Peak III	63.4	0.0	1.82

Results quality –Good

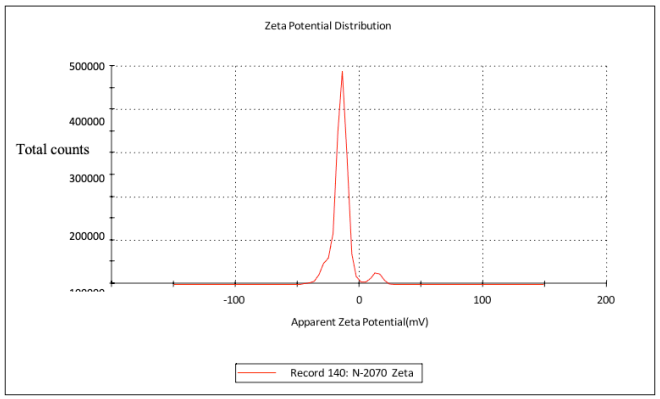

Figure: 5 Zeta Potential Distributions

The zeta potential (ZP) profile of ABK nano particles at different count and apparent zeta potential is shown in figure 5. The value of the zeta potential of ABK is -13.6mV.The negative zeta potential value shown by ABK as due to possible capping of the bio organic compounds present in the extract. The stability of the formulation was evaluated by measuring zeta size distribution and zeta potential. The zeta potential of test formulation was determined in water as dispersant. The zeta potential value far from zero indicates that the particles disperse well in the media since the

electrostatic repulsive force among the particle is large and thus, the particles have high aqueous stability. Thus the nano particle dispersion with a value of a zeta potential far from zero was stable or relatively mono disperse, while that a value close to zero indicate poor mono dispersity. (35) Surface modification by citrate could prevent particle agglomeration and enable dispersion of individual particle in the media. Zeta potential is the electrical potential at the solid liquid interface in response to the relative movement of the particle and solvent. The potential was evaluated by dispersing the drug in double distilled water. ZP value is a good indicator of particle electrical potential. The charge of the material results from the change on the particle surface, the reduction of surface energy is due to reduction in the particle size as negative value –13.6mV indicating greater surface potential; this was supported by floatability of ABK powder. The negative zeta potential value of ABK shows stability of formulation and possible capping of the bioorganic compound present in the powder.

BET analysis attributed to highest surface area 137.671m^2/g,(pore size 3.8752nm and pore volume 0.090956 cm^3/g) which increased surface area are expected to improve the bioavailability of ABK formulation. It is evident from Fig 6 that the ABK sample should excellent adsorption character with minimal hysteresis. The analysis by BET indicates the precise specific surface area evaluation of materials by nitrogen multilayer adsorption measured as a function of relative pressure using a fully automated analyzer. The technique circumscribe external area and pore area evaluations to determine the total specific surface area in m^2/g yielding important information in studying the effects of surface porosity and particle size in many applications

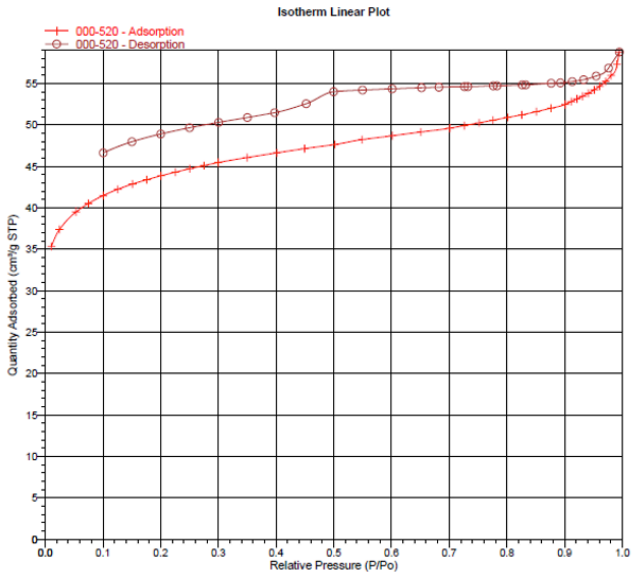

Figure: 6 Isotherm linear plots

Conclusion

The consistency, fineness and tactile sensation test results shows the ABK is non-adhesive, minute particle size which enhances absorption and assimilation of particle in body without producing any irritation to the mucous membrane of gastrointestinal tract (GIT). The parameters like freeness, fineness and lightness confirm the excellent finishing stage of ABK. The Modern method of characterization revealed the ABK has long shelf life, prevent microbial growth.ABK powder has low porosity and good compaction and poor flowing rate indicate the greater inter-particular interaction which endorse the good character and excellent finished product. Organic molecule present in the ABK interacted with water on zeta size analyser. Therefore the size was found to be micrometric level in zeta sizer. The negative zeta potential value of ABK shows stability of formulation and possible capping of the bioorganic compound present in the powder. Increased surface area is expected to improve the bioavailability of ABK formulation. Hence the study confirms the ABK efficacious consumable medicinal product for human beings and suitable for the treatment of Anaemic conditions.

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