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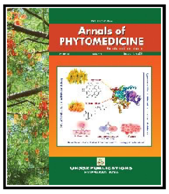

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# Characterization and elemental analysis of Kushta-e-Sadaf (Calcinated oyster shell)

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**Abstract**

The drug known as Kushta-e-Sadaf, which is produced from calcined oyster shells, has been acknowledged in traditional medicine for the alleged health advantages it offers. In addition to performing an elemental analysis, the purpose of this study is to describe the physical and chemical properties of Kushta-e-Sadaf and to gain an understanding of its composition and the possible uses it may have. The structural integrity of the material as well as the elemental elements of the material was analyzed using techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). It has been shown through preliminary research that a substantial amount of calcium carbonate was present, in addition to trace components that may be responsible for its therapeutic qualities. This discovery makes a significant contribution to a more in-depth knowledge of Kushta-e-Sadaf, therefore clearing the path for its application in a variety of sectors, including medicine and materials science.

## 1. Introduction

The ancient preparation known as Kushta-e-Sadaf, which is prepared from calcined oyster shells, continues to play a significant role in a wide range of medicinal treatments, particularly within the branches of medicine known as the Ayurveda and Unani medicine. Throughout human history, this preparation has been utilized to reap the purported health advantages that it provides. These benefits include the improvement of bone density and the relief of gastrointestinal diseases. In the process of making Kushta-e-Sadaf, one of the steps involves the calcination of oyster shells. The physical and chemical properties of the shells are altered as a result of this technique, which ultimately has an impact on the product's efficiency and its ability to be utilized.

The utilization of oyster shells by individuals from a wide range of civilizations has occurred over the course of several centuries, mostly because oyster shells include both nutritional value and natural medicinal properties. One of the reasons why Kushta-e-Sadaf is held in such high esteem in traditional medicine is because of the significant amount of calcium it contains. A wide range of biological processes, such as muscle contraction, nerve impulses, and the coagulation of blood, all require calcium in order to function properly. It is common practice to prescribe Kushta-e-Sadaf for the treatment of conditions such as osteoporosis, which requires a higher calcium intake to maintain bone density. The benefits of Kushta-e-Sadaf for medicinal purposes extend far beyond the basic act of providing calcium supplements (Lev, 2010).

Kushta-e-Sadaf has been asserted to contain antacid properties, which enables it to be used effectively in the treatment of acid reflux and other gastrointestinal conditions. In addition to this, Kushta-e-Sadaf also works to improve bone health. The mineral composition of this substance, which is made up mainly of calcium carbonate, adds to its ability to neutralize stomach acidity. In addition to providing relief to those experiencing hyperacidity and the symptoms associated with it, this substance also has the ability to neutralize other symptoms.

Most Kushta-e-Sadaf is made up of calcium carbonate ($CaCO_3$), which is a significant amount of the calcined oyster shell. Because calcium carbonate is the most abundant component, this is the situation that has materialized. This preparation undergoes a thermal breakdown when it is exposed to calcination, which ultimately leads to the creation of calcium oxide (referred to as CaO). The following reaction of calcium oxide with water might result in the formation of calcium hydroxide $Ca(OH)_2$. The significance of this shift lies in the fact that calcium hydroxide is utilized in a wide variety of applications, not only in the industrial sector but also in the medical sector.

In addition to calcium, it is probable that Kushta-e-Sadaf includes trace elements as potential components. These trace elements consist of magnesium, potassium, and phosphorus, in addition to a wide range of minerals that are essential for the health of the human body as a whole. These components, which have the capacity to play an important role in metabolic processes, have the potential to enhance the overall therapeutic efficacy of the preparation. Furthermore, it is likely that this improvement will be implemented. The inclusion of trace elements such as these is particularly relevant because they

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have the ability to contribute synergistically to the health benefits that are associated with the consumption of Kushta-e-Sadaf (Makbul *et al*., 2018).

Although Kushta-e-Sadaf has been used for its potent medicinal properties for many years, there is a growing desire for scientific validation of its traditional use. Using modern tools, researchers can not only able to explore the composition of Kushta-e-Sadaf, but they are also able to investigate its various modes of action and its bioavailability that are associated with it.

In order to provide a description of Kushta-e-Sadaf, several innovative approaches are applied. Scanning electron microscopy (SEM) for morphological examination, and energy-dispersive X-ray spectroscopy (EDS) for detecting the elemental composition of the substance are some of the methods that fall under this category. When these methods are utilized, it is feasible to acquire a comprehensive understanding of the material at both the macroscopic and microscopic levels. When one has this information, they are able to get insights into the material's physical features as well as possible interactions within biological systems (Makbul *et al*., 2017).

The primary objectives of this study are to characterize the physical and chemical properties of Kushta-e-Sadaf using various analytical techniques, and to conduct an elemental analysis to determine the composition and concentration of key elements present in the preparation. Through the achievement of these objectives, the research strives to fill in the gaps that have been identified in the current literature regarding Kushta-e-Sadaf (Sudha *et al.,* 2009).

Its purpose is to offer a scientific foundation for its conventional applications and to investigate new pathways for its application. To validate the function that Kushta-e-Sadaf plays in health and wellbeing, as well as its potential contributions to disciplines such as materials science and environmental sustainability, it is vital to have a solid understanding of the elemental makeup and characterization of this substance.

## 2. Materials and Methods

In this section, the materials and methods that were utilized in the characterization and elemental analysis of Kushta-e-Sadaf (calcined oyster shell) are discussed in detail. Particular attention is paid to the analysis of particle size; scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS), and inductively coupled plasma mass spectrometry (ICP-MS).

### 2.1 Particle size analysis

The particle size has a prominent influence on the drug dissolution rate, bioavailability of the drug, a smaller particle size has better absorption and solubility which also impacts the drug stability and uniformity of the formulation (Jyoti and Anoop, 2024). The particle size of the Kushta-e-Sadaf was determined by photon correlation spectroscopy using Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK). The formulation was diluted with distilled water and filtered through a 0.22 µm membrane filter in order to eliminate multiscattering phenomena and experimental errors. Light scattering was monitored at 25°C at a scattering angle of 90°C (Akhtar *et al.,* 2016). Additionally, a laser diffraction particle size analyzer was utilized to achieve more accurate measurements of particles with a finer particle size. An appropriate medium was used to scatter the sample and then it was examined, resulting in the production of detailed information on the mean particle size and span. A full understanding of the particle size distribution in Kushta-e-Sadaf was made possible by the data obtained from both approaches (Ali *et al.,* 2017).

### 2.2 Elemental analysis by scanning electron microscopy (SEM)-energy dispersive X-ray spectroscopy (EDS)

SEM gives point-by-point imaging of a material's surface at the micro- and nanoscale, offering bits of knowledge into primary highlights, for example, grain size, porosity, and cracks (Giannuzzi, 2018). EDS identifies and evaluates components present in a sample by determining the distinct X-rays radiated by electron beam interactions (Sriram *et al.,* 2023). This makes it valuable for recognizing obscure materials and concentrating on basic conveyance (Rohit *et al.,* 2024). This method has also been found to be beneficial in forensic and Archaeological Investigations (Bell, 2012; Scott, 2017), as well as in Environmental and geological research, semiconductor, and Nanotechnology Studies (Reimer, 2013). So, on the basis of these application, SEM-EDS has been used for the elemental analysis of Kushta-e-Sadaf.

The morphological and elemental analysis of Kushta-e-Sadaf was performed with the assistance of energy-dispersive X-ray spectroscopy (EDS) in conjunction with SEM. To prepare the sample, a tiny quantity of Kushta-e-Sadaf was first put on a metal stub by means of conductive double-sided tape. Subsequently, a thin layer of gold or carbon was coated on the metal stub to improve the conductivity of the sample and reduce the charging effects that occurred during imaging. After that, the sample was inspected using the SEM instrument at an accelerating voltage that was generally between 5 and 15 kV. High-resolution photographs of the surface morphology were obtained, which allowed for the collection of information on the shape, texture, and structural characteristics of the particles. After imaging, EDS analysis was performed in the same areas in order to ascertain the specific elemental composition of the sample. The X-ray spectra that corresponded to the elements that were present were created by the EDS system, which made it possible to quantify the amounts of those elements. In addition to providing vital information on the elemental composition of Kushta-e-Sadaf, combined data from SEM and EDS data also provided information on the physical features of the target (Tariq *et al.,* 2014; Sultana *et al.,* 2024; Gupta *et al.,* 2024).

### 2.3 ICP-MS analysis

After a comprehensive elemental analysis of Kushta-e-Sadaf was performed, inductively coupled plasma mass spectrometry (ICP-MS) was applied. It is a sophisticated analytical method used for the identification and quantification of trace elements present in a sample. ICP-MS can identify elements at parts per trillion (ppt) levels, making it a powerful tool for environmental, pharmaceutical, and clinical studies (Hou and Jones, 2000). For this, a digestion vessel was filled with a mixture of strong nitric acid and hydrochloric acid in a ratio of 3:1. This was followed by the addition of approximately 0.5 grams of the sample, which was then accurately weighed and deposited within the vessel. To achieve thorough dissolving, this combination was heated on a hot plate or digested in a microwave until it was completely dissolved. It was ensured that the sample concentration was within the operating range of the ICP-MS by diluting the solution with deionized water to a specified volume after the digestion process was completed and the solution had cooled down. Following the instructions provided by the manufacturer, the ICP-MS was calibrated using calibration standards that had known amounts of the elements that were the focus of the analysis. Subsequently, the sample solution that had been diluted was injected into the instrument for analysis (Makbul *et al.,* 2018). The calibration curves that were developed from the standards were used to determine the right concentrations of the various elements that were present in Kushta-e-Sadaf. Additionally, quality control procedures, such as measurement replicates and blank samples, were used to evaluate the detection limits, precision, and accuracy of the sample. Full knowledge of the elemental concentration of Kushta-e-Sadaf was obtained by using this methodical approach to elemental analysis, which subsequently informed its possible uses in a variety of sectors (Aqeel *et al.,* 2023; Shariq *et al.,* 2022).

## 3. Results

Kushta-e-Sadaf was subjected to particle size analysis, SEM-EDS analysis, and ICP-MS analysis, and the results of these studies are shown in this section. In the interest of clarity, the data have been grouped into tables and graphs.

### 3.1 Particle Size Analysis

Particle size analysis was covered by suspending the drug in 0.5%, 1% and 1.5% CMC because an optimal concentration of suspending agent (CMC) is crucial for controlling the effective particle size of any suspension (Fedina *et al.,* 1997; Nyandoro *et al.,* 2019).

### 3.1.1 0.5% CMC

The particle size analysis of Kushta-e-Sadaf suspended in 0.5% carboxymethylcellulose (CMC) revealed three distinct peaks, indicating a diverse particle size distribution within the sample. The first peak, at 498.5 nm, represented 91.1% of the intensity, signifying it as the dominant particle size. This suggests that a substantial proportion of the particles are relatively large, which could indicate the presence of aggregates or clusters of smaller particles, a common occurrence in natural materials. The high intensity associated with this peak reflects its significant contribution to the overall scattering profile of the sample. The second peak, at 36.22 nm, represented a smaller fraction of the particle population, contributing only 8.9% to the intensity. This indicates that although these smaller particles are present, they are not as prevalent as the larger ones. The relatively low intensity suggests that these finer particles may enhance the functional properties of Kushta-e-Sadaf by increasing its surface area for interaction in various applications. The third peak, showing a size of 0.000 nm and an intensity of 0.0%, indicates that no particles were detected in this size range, implying a lack of ultrafine particles or that any in this category are below the detection limit.

The standard deviations associated with each peak provide insight into the distribution and uniformity of the particle sizes. The standard deviation of 162.6 nm for Peak 1 suggests a relatively broad size distribution, indicating variability among the larger particles. On the contrary, the smaller standard deviation of 7.532 nm for Peak 2 indicates that the smaller particles are more uniform in size (Table 1 and Figure 1).

**Table 1: Particle size for formulation 0.5% CMC**

| Peak | Size (d.nm) | % intensity | St. Dev. |
|---|---|---|---|
| Peak 1 | 498.5 | 91.1 | 162.6 |
| Peak 2 | 36.22 | 8.9 | 7.532 |
| Peak 3 | 0.000 | 0.0 | 0.000 |

*d.nm- diameter nanometer

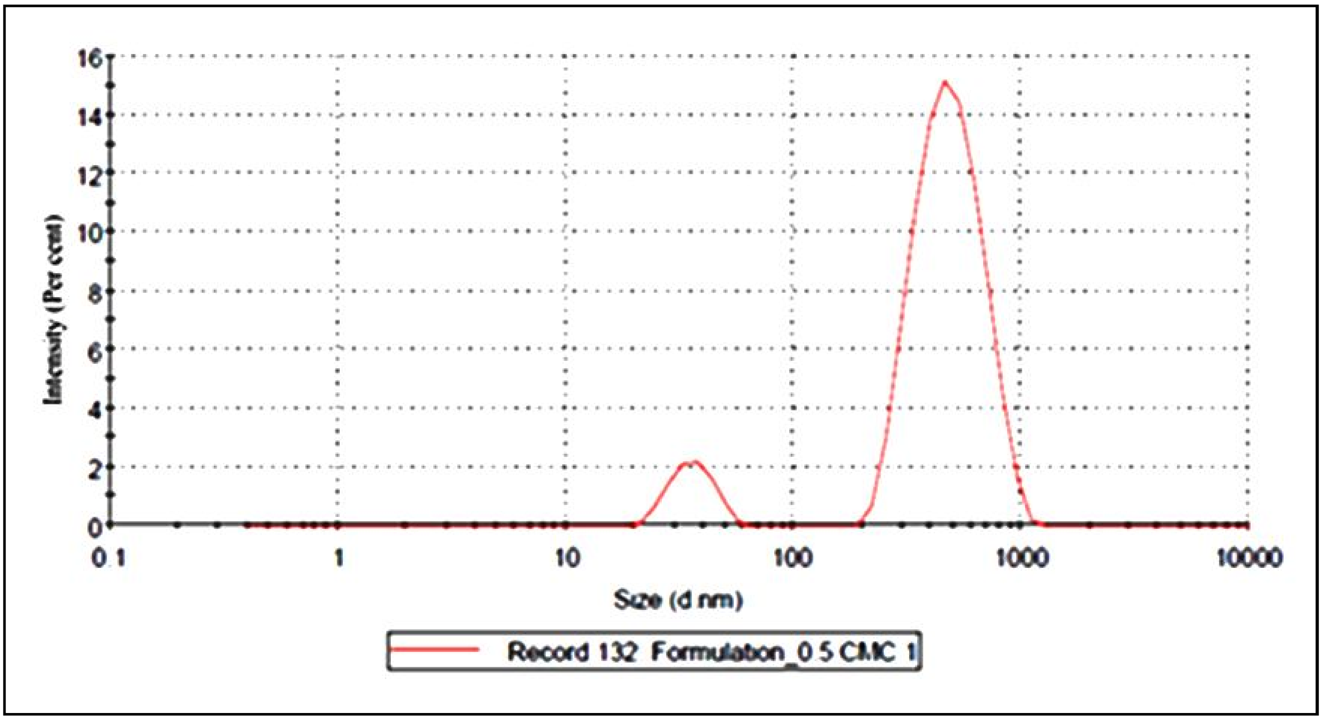


**Figure 1: Particle size for formulation 0.5% CMC.**

In general, the analysis reveals that Kushta-e-Sadaf consists predominantly of larger particles, with a significant presence of smaller particles, which may influence its physical properties and potential applications in areas such as supplementation and medicinal formulations. Particle size plays an important role in different pharmacological aspects, such as bioavailability, smaller particle size shows increased bioavailability as smaller particles have a larger surface-to-volume ratio, which facilitates their dissolution rate, pharmacokinetic properties such as ADME, and onset of action of the smaller particle size drug is more, duration of action was found to be lower in smaller particle size drugs as they are readily eliminated (Ha *et al.,* 2024). Understanding these characteristics is essential to optimize their use across various industries. Here, in this formulation, it was found that the average size of the particles was 302.4 d.nm with polydispersity index of 0.523.

### 3.1.2 1% CMC

The particle size analysis of Kushta-e-Sadaf suspended in 1% carboxymethylcellulose (CMC) produced three distinct peaks, indicating a varied particle size distribution. The first peak, measuring 911.1 nm, was the most prominent, contributing 90.9% to the total intensity. This suggests that a significant portion of the particles in the sample are relatively large, with high intensity indicating their substantial role in the overall scattering profile. The large size of these particles may suggest the formation of aggregates, which is common in natural materials and could influence their behavior in various applications. The second peak, at 76.59 nm, represented a much smaller portion of the particle population, contributing only 9.1% to the intensity. This indicates that, while smaller particles are present, they are less abundant compared to the larger ones. The relatively low intensity of this peak suggests that these finer particles could potentially enhance the functional characteristics of Kushta-e-Sadaf, such as increasing surface area for better interaction in applications such as supplementation or medicinal formulations. The third peak, showing a size of 0.000 nm with an intensity of 0.0%, indicates that no particles were detected in this range, implying the absence of ultrafine particles or that any present particles are below the detection limit. The standard deviations provide additional information on the size distribution. The standard deviation for Peak 1 was 299.9 nm, indicating a broad size distribution and considerable variability between the larger particles. On the contrary, the smaller standard deviation of 16.40 nm for Peak 2 indicates a more uniform size among the smaller particles (Table 2 and Figure 2).

**Table 2: Particle size for formulation 1% CMC**

| Peak | Size (d.nm) | % intensity | St. Dev. |
|---|---|---|---|
| Peak 1 | 911.1 | 90.9 | 299.9 |
| Peak 2 | 76.59 | 9.1 | 16.40 |
| Peak 3 | 0.000 | 0.0 | 0.000 |

Overall, the analysis shows that Kushta-e-Sadaf in a 1% CMC suspension contains predominantly larger particles, with a minor presence of smaller particles. This size distribution may significantly influence its physical properties and potential applications, highlighting the need for further exploration of its use in various industries. Understanding these characteristics is crucial to optimizing the formulation and application of Kushta-e-Sadaf in different contexts. Here, in this formulation, it was found that the average size of the particles was 514.3 d.nm with polydispersity index of 0.634.

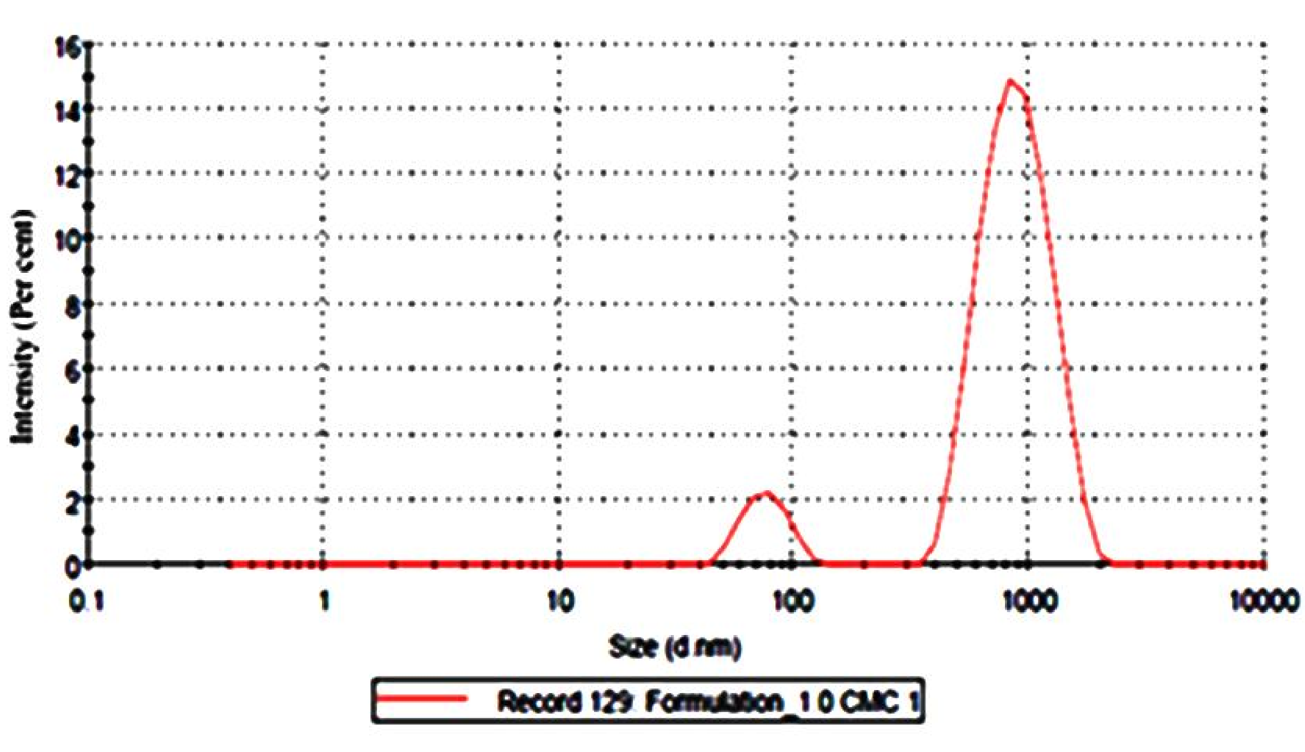


**Figure 2: Particle size for formulation 1% CMC.**

### 3.1.3 1.5% CMC

The particle size analysis of Kushta-e-Sadaf suspended in 1.5% CMC revealed three distinct peaks, indicating a diverse range of particle sizes. The first peak, at 1202 nm, was the most significant, contributing 82.0% to the total intensity. This high intensity suggests that a considerable portion of the particles in the sample are relatively large. The substantial size of these particles may indicate the presence of aggregates or clusters, which can influence the behavior and functionality in various applications. The second peak, measuring 120.3 nm, accounted for 11.6% of the intensity. This indicates that although these smaller particles are present, they are not as dominant as the larger ones. The relatively low intensity of this peak suggests that although these particles contribute less to the overall scattering profile, they may enhance specific properties of Kushta-e-Sadaf, such as surface area and reactivity, which can be beneficial in applications such as supplements or medicinal formulations. The third peak, at 27.20 nm, contributed 5.2% to the intensity, indicating a minor presence of even smaller particles. The standard deviation for this peak was 5.745 nm, suggesting that these smaller particles are relatively uniform in size, which may be advantageous for certain applications where consistency is important. Standard deviations for the larger peaks reveal additional insights into the particle size distribution. The first peak had a standard deviation of 753.8 nm, indicating considerable variability among the larger particles, while the second peak had a smaller standard deviation of 32.86 nm, reflecting a more consistent size among the smaller particles (Table 3 and Figure 3).

Overall, the analysis demonstrates that Kushta-e-Sadaf in a 1.5% CMC suspension consists primarily of larger particles, with a notable presence of smaller particles as well. This size distribution could significantly impact its physical properties and potential applications, emphasizing the importance of further investigation of its uses across various fields. Understanding these characteristics is essential to optimize the formulation and application of Kushta-e-Sadaf in different contexts. Here, in this formulation, it was found that the average size of the particles was 514.3 d.nm with polydispersity index of 0.634.

**Table 3: Particle size for formulation 1.5% CMC**

| Peak | Size (d.nm) | % intensity | St. Dev. |
|---|---|---|---|
| Peak 1 | 1202 | 82.0 | 753.8 |
| Peak 2 | 120.3 | 11.6 | 32.86 |
| Peak 3 | 27.20 | 5.2 | 5.745 |

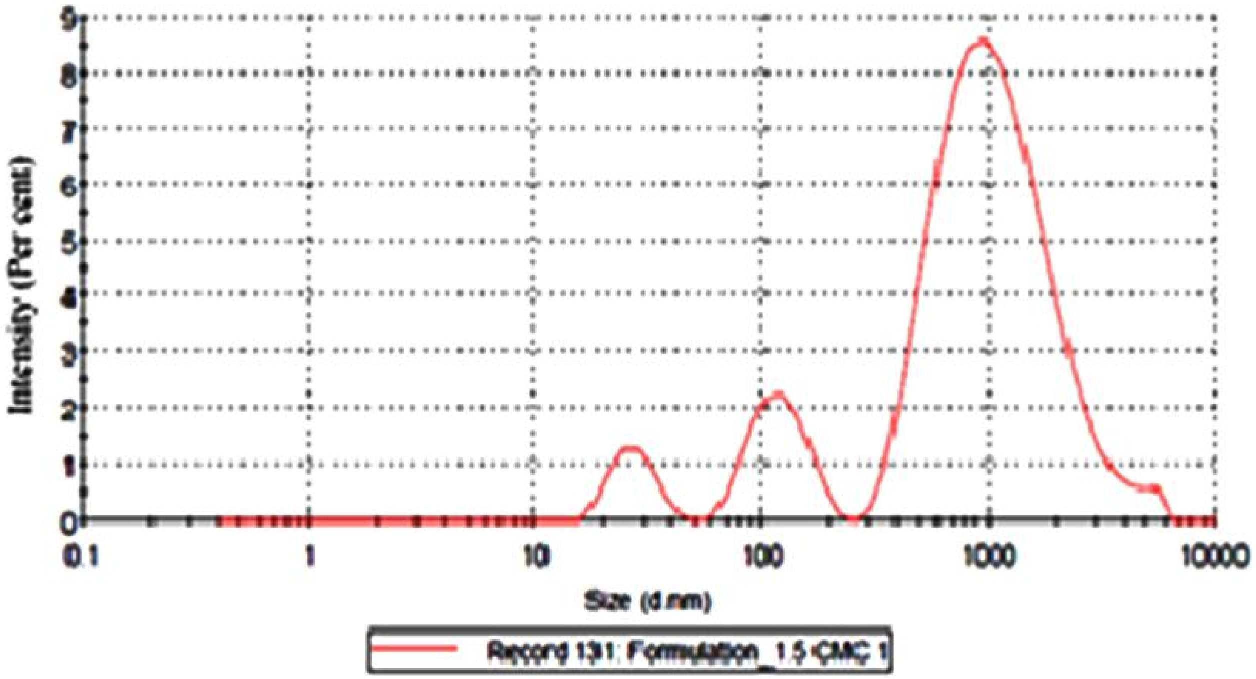


**Figure 3: Particle size for formulation 1.5 % CMC.**

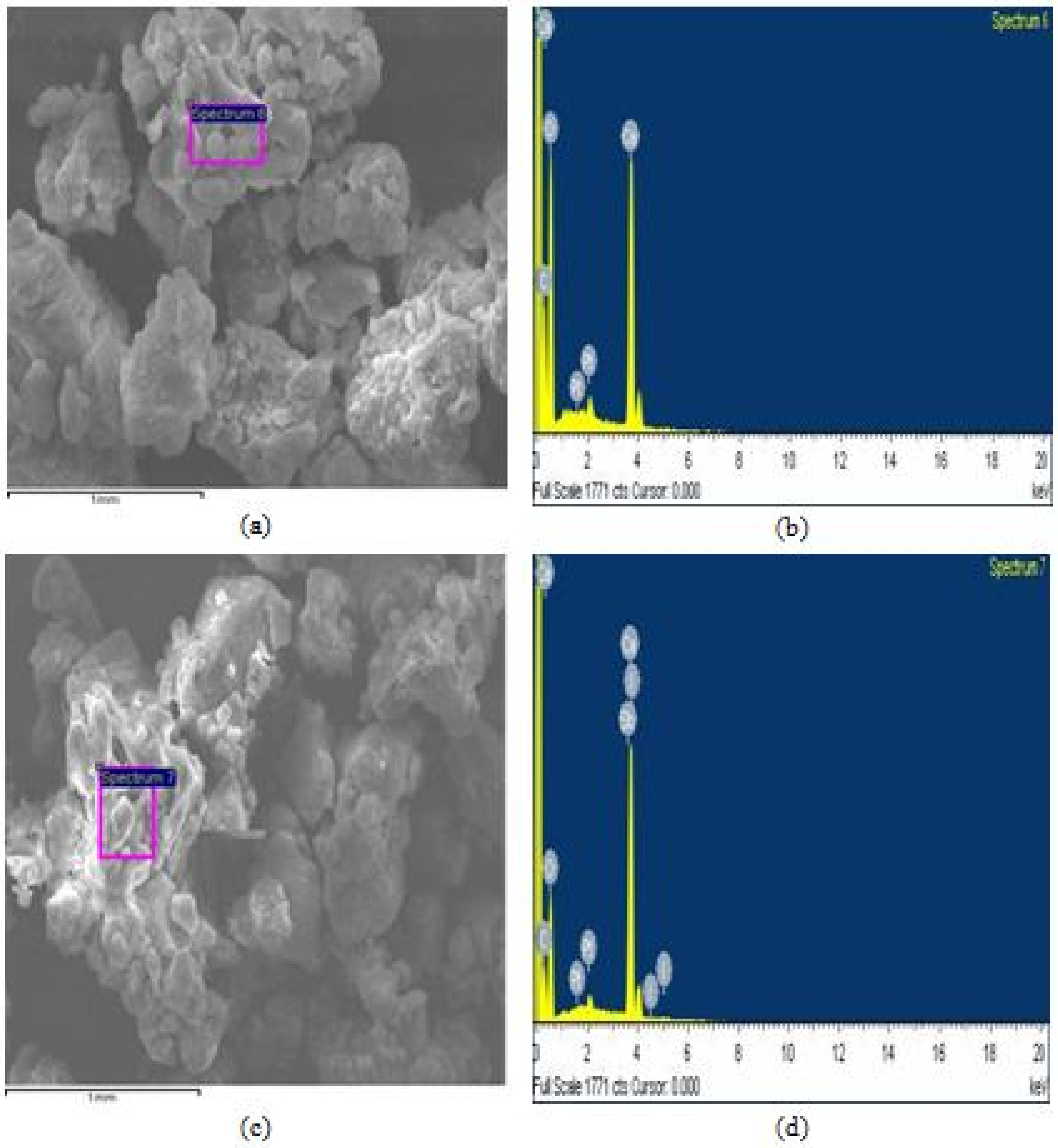
### 3.2 Elemental analysis by SEM- EDS

Scanning electron microscopy with Energy Dispersive Spectroscopy (SEM-EDS) is diversely used in medicine for the assessment of morphology and composition of active pharmaceutical ingredients and excipients, found in any drug formulation. It has been used in the identification of impurities, biocompatibility of various pharmaceutical products, which proves to be a potent tool in the research field (Löbenberg and Amidon, 2000; Zhang *et al.,* 2008; Vippagunta *et al.,* 2001; Rezwan *et al.,* 2006). In the SEM-EDS study, a specific area or point where the composition of the element has been measured is assigned a spectrum number. Here, in this study, spectrums 6 and 7 have been assessed.

In order to confirm the presence of calcium element in Kushta-e-sadaf, EDS analysis was performed. During the EDS measurement, different areas are focused and the corresponding peaks are shown in Figure 4. Calcium is found to be present in the drug in both EDS spectrum. In spectrum 6, the amount of calcium was 5.73 and in spectrum 7 it was found to be 10.93 measured in atomic percent for calcium. Details of both the EDS spectra for calcium measured in atomic and weight % are given in Table 4.

**Table 4:** EDS weight ratio of calcium element (Ca, K) in Kushta-e-Sadaf using two spectrums (spectrums 6 and 7)

| Ca, K element | Weight% | Atomic% |
|---|---|---|
| **Spectrum 6** | 13.95 | 5.73 |
| **Spectrum 7** | 22.18 | 10.93 |



(a) (b) (c) (d)

**Figure 4:** (a) SEM of Kushta-e-sadaf at spectrum 6, (b) EDS at spectrum 6, (c) SEM of Kushta-e-sadaf at spectrum 7 and (d) EDS at spectrum 7.

**Table 5: Concentrations of elements in Kushta-e-Sadaf (ppb)**

| S.No. | Element | Concentration [ppb] |
|---|---|---|
| 1 | Calcium (Ca)$^{43}$Ca [He] | 224547.956 |
| 2 | Calcium (Ca)$^{44}$Ca [He] | 358174.004 |
| 3 | Magnesium (Mg)$^{24}$Mg [No Gas] | 2403.556 |
| 4 | Copper (Cu)$^{63}$Cu [He] | 56.369 |
| 5 | Zinc (Zn) | 4462.147 |
| 6. | Trace elements | Varies |

### 3.3 ICP-MS analysis

Inductively coupled plasma mass spectrometry (ICP-MS) plays an important role in pharmacology and biomedical research, particularly in the detection of elemental impurities of heavy metals such as lead (Pb), arsenic (As), mercury (Hg) and cadmium (Cd) in drug preparations (Rudovica *et al.,* 2014), in the bioavailability of metal containing drugs like platinum in chemotherapy (Harrington and Taylor, 2015), and in the development of biomarkers and personalized medicine in various metal toxicity induced diseases like in neurodegenerative disorders, cancer and metabolic disorders (Zhang *et al.,* 2018).

Here, in this study, essential elements such as calcium, magnesium, copper, and zinc were found in the concentration as given in Table 5. Also, we have used two isotopes for the estimation of calcium concentration, *i.e.*. $^{43}$Ca and $^{44}$Ca although $^{40}$Ca is abundant but due its isobaric interference with $^{40}$Ar, high background noise, and better alternatives like $^{43}$Ca and $^{44}$Ca it is not used. $^{43}$Ca and $^{44}$Ca have shown a beneficial role in the study of bone metabolism, calcium and found to be a biomarker of osteoporosis and other bone-associated disorders (Shroff *et al.,* 2020).

## 4. Discussion

For the purpose of providing a complete characterization of Kushta-e-Sadaf, the findings of this investigation have revealed significant insight into the chemical and physical characteristics of the substance involved. Based on the results of the particle size study, it was determined that most of the particles are located within the range of 125 to 250 µm, although there is also a considerable fraction of particles that are finer. The low PDI indicates that the particle size was uniform (Ali *et al.,* 2023). This size distribution is significant because it has the potential to be applied in a variety of disciplines, such as drugs and nutritional supplements, where the size of the particles can have an effect on the bioavailability and absorption rates of the substance.

Based on the results of the SEM-EDS study (Figure 4) and ICP-MS (Table 4), it was determined that calcium is the predominant element present, accounting for approximately 65% of the components. This high calcium concentration highlights the fact that Kushta-e-Sadaf has traditionally been used as a calcium supplement. There is a possibility that the therapeutic effects of this molecule are due, in part, to the presence of other elements such as magnesium, copper, and zinc. This lends credence to the idea that this element presents a wider range of health advantages than previously acknowledged.

Like calcium, it has a major benefit in bone health, neurological health, blood clotting, cardiovascular functions, and immune deficiencies. It also plays a crucial role in the prevention of osteoporosis and hypertension (Reid *et al.,* 2014; Griffith *et al.,* 1999; Del Valle *et al.,* 2011). Copper being a trace element, it is involved in red blood cell production, immune function, and antioxidant defense activities (An *et al.,* 2022; Klevay, 2000; Scheiber *et al.,* 2014). Magnesium is important for muscle and nerve function, cardiovascular health, and glucose metabolism (Kass *et al.,* 2012; Barbagallo and Dominguez, 2015; Mauskop and Varughese, 2012). Zinc plays a crucial role for immune function, wound healing, antibacterial activity, and enzymatic reactions (Prasad, 2008; Lansdown *et al.,* 2007; Deshmukh *et al.,* 2022; Takeda, 2001).

## 5. Conclusion

The ICP-MS results provided quantitative data on the elemental concentrations, revealing exceptionally high levels of calcium (224547.956 ppb) and notable amounts of magnesium, copper, and zinc. These findings support the idea that Kushta-e-Sadaf is a rich source of essential minerals, which can be beneficial for various physiological functions, particularly in bone health and metabolic processes. The characterization of Kushta-e-Sadaf through particle size analysis, SEM-EDS, and ICP-MS has established a solid scientific foundation for its traditional uses. The findings validate its potential as a valuable source of calcium and other trace elements, which may contribute to its effectiveness in traditional medicine. Future research will explore the bioavailability and physiological effects of Kushta-e-Sadaf, as well as its potential applications in modern health products and industries. The insights gained from this study not only affirm the historical significance of Kushta-e-Sadaf but also open avenues for further exploration of its medicinal properties and applications.

## 6. Implications for future research

The results of this study have the potential to open the path for more research into Kushta-e-Sadaf and other natural substances that have comparable properties. Researchers have the ability to facilitate a greater acceptance of traditional treatments in current healthcare practices by providing empirical evidence to support historical claims that have been made. Additionally, gaining knowledge of the characteristics of Kushta-e-Sadaf could result in the development of novel applications in a variety of industries, such as the pharmaceutical industry, the cosmetics industry, and the food technology industry (Kapoor, 2010). As the desire for natural and organic products continues to increase, it is expected that the significance of Kushta-e-Sadaf in the realm of health and wellness will also continue to improve. This research not only intends to confirm ancient methods but it also intends to stimulate future inquiries into the numerous advantages of substances that are derived from natural sources.

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## Conflict of interest

The author declares no conflicts of interest relevant to this article.

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