INDUSTRIAL APPLICATION SUITABILITY OF INDIGENOUS BENTONITE CLAY IN PHARMACEUTICAL SECTOR

By

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Thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy

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ABSTRACT

In this PhD thesis the pharmaceutical suitability study of bentonite, the most important clay with wide spread industrial applications, is carried out. Bentonite is an exciting and truly amazing natural material varies in composition and texture from place to place. Hence, not equally well in performance for its specific industrial uses. They are mainly composed of a montmorillonite and other minor impurities that give them a unique “fingerprint”. The amount of pure montmorillonite in bentonite is key to its purity and performance. Bentonite can be considered as raw pharmaceutical materials that once properly identified, evaluated and/or modified to fulfil regulatory pharmacopoeial requirements and mineral purity. No such work has been done on Pakistani bentonite and no published data is available. In this work, the mineralogical, microbiological, geochemical and physicochemical studies of raw samples collected from three Pakistani mining sites located at Shagai (district Karak), Dag Ismial Khel (district Nowshera) and Chandan Garhi (district Peshawar) is carried out.

The bentonites sample collected were purified by three different methods i.e. simple sedimentation using sodium hexametaphosphate as a dispersing agent in Method-I, classical NaCl treatment followed by sedimentation in method-II and activation by Na₂CO₃ at various ratio (2, 3, 5, 8 g Na₂CO₃/100 g bentonite) followed by sedimentation technique in Method-III.

The raw samples and the samples purified by three methods mentioned above, were characterized by X-ray diffraction (XRD), Scanning Electron Microscope (SEM), X-ray Fluorescence (XRF), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), N₂ Adsorption-desorption Isotherm at Temperature 77.35K, Microbial test, CEC measurements, Pharmacopeial tests (i.e. pH, swelling capacity, gel formation) and laser granulometry. The test results obtained for raw and purified bentonite from each site were compared among them as well as with others.

The mineralogical and chemical composition study revealed that all the samples from the three sites were of high quality bentonite, containing mainly Ca⁺². 
montmorillonite with minor contents of illite and quartz. In addition, kaolinite was found in Karak, albite in Peshawar and Nowshera bentonite in small quantity. The swelling volume (apparent volume of 2g clay/100ml of distilled water) of all the three raw bentonite were in the range of 5mL to 7mL which is less than pharmacopeias requirements i.e. ≥ 20 mL. The gel formation value (4mL) of Peshawar bentonite also failed to comply with the pharmacopeias requirement (≤2mL).

All the purified samples were found free of quartz impurities as confirmed by XRD. The swelling, gelling and other properties were also improved by the purification process. The XRD and CEC tests confirmed the conversion of raw bentonite to sodium bentonite by 5% Na₂CO₃ for Karak and Nowshera while that of Peshawar by 3% Na₂CO₃. Moreover, XRD, XRF and TGA studies also testify the conversion of calcium bentonite to Sodium form by method-II. The results for purified samples from the three sites are in conformity with major pharmacopeial requirement of advanced countries for bentonite, hence Pakistani bentonite in purified can be designated as value added pharmaceutical raw material.

The comparative study of purified samples revealed its variation in properties quantitatively, which make them important for specific pharmaceutical uses. The results of samples purified by three different methods are also varying from each other. All the studied bentonites in purified form could be used as suspending agent for both oral and topical application but the sample obtained by Method II and III could be preferred because of its excellent swelling capacity and sedimentation volume. The high CEC, high surface area, small particle size and pore size distribution suggesting their use as a good adsorbent of drugs and a drug carrier in control release of medicine.
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THESIS ORGANIZATION

This thesis consists of four chapters, and the contents of each chapter are explained as follows:

CHAPTER 1: This introductory chapter presents the significance of this study, study area, problem statement, objective and scope of study.

CHAPTER 2: In this chapter theoretical study of denomination and specification of bentonite, literature reviews related to bentonite clay use in industrial application especially in pharmaceutical sector were discussed. The attractive properties of bentonites due to which this natural material play an important role in the business world were also discussed.

CHAPTER 3: This chapter describes the experimental procedure namely material (bentonite samples), purification process i.e. simple sedimentation using sodium hexametaphosphate as dispersing agent in Method-I, sodium chloride treatment followed by sedimentation in Method-II and soda activation to get pure sodium bentonite in method-III, sample preparation methodology and the characterization techniques.

CHAPTER 4: In this chapter we evaluated and analyzed the results and discussion of raw and purified samples of local bentonite clay. We also summarized the main process required to purify the bentonite from local sites and conclusive remarks on pharmacopeias requirement
CHAPTER -1

INTRODUCTION

1.1 General Overview

Clay is the most important, plentiful, and low cost naturally occurring material, widely used in diverse fields that are agriculture, engineering, construction, process industries and environmental application [1]. Clay composed primarily of fine-grained minerals, which generally show plastic behavior at appropriate water contents and hardness upon drying or firing [2]. Clays are studied by, and used in many disciplines for fundamental, applied research and in industry. The multidisciplinary approach is at the border between material science and colloidal science [3]. The rapid development in the use of clays and clay minerals in the production of enhanced materials and the need for optimization of clays for hundreds of uses, including the area of health, is the consequence of endeavor of clay science which combining chemistry, colloid science, material science, environmental science, physics, soil science, civil engineering, geotechnical engineering and biological science [3, 4].

The term bentonite, which is very frequent in the business world is used to designate any plastic, colloidal, swelling clay dominated by one of the largest groups of clay minerals called smectite (such as montmorillonite, nontronite, beidellite, saponite and hectorites) with no regards for its origin [1, 5-6]. Illite” The member of clay mineral group called mica” is often found in bentonite as a minor clay mineral impurity. Non-clay mineral found in bentonite are zeolites, feldspars, carbonates,
gypsum, and crystalline silica (quartz and opals) [7-9] Commercial bentonite contains either calcium montmorillonite (Ca$^{2+}$-montmorillonite) or sodium montmorillonite (Na$^{+}$-montmorillonite) and lower amount of other minerals [6]. Bentonite are extensively used in foundry sand as a binder, in paper industry, drilling mud, catalysis, barrier for water and nuclear waste, iron ore pellets, ceramics and refectories, rubber, cement, soil stabilization, cat litter, perfume, cosmetic, plastic, paint, food and pharmaceutical [1]. In pharmaceutical industry bentonite is commonly used as medicinal product as an excipient and active ingredient in the forms of liquid (suspensions, emulsions), semisolid (creams, ointments) and solid (capsules, tablets and powders) either for topical or oral administration [10-15]. The usefulness of the bentonite due to which it is considered as a suitable pharmaceutical raw material are high surface area, small particle size, porous nature, high swell potential, gel formation, alkaline nature, high cation exchange capacity low or null toxicity for the patient and low price [16-19].

The world wide raw material, bentonite, also occurs in Pakistan in a large scale. According to British geological survey, the Pakistani bentonite production increases year by year, in 2007 the total bentonite production was 33177 tones [20]. Though bentonites found abundantly in Pakistan but none of them have yet been properly characterized or investigated for pharmaceutical application. Therefore prior to its use, it is crucial importance for indigenous bentonite to be evaluated and/or purified, modified by different chemical treatment to comply with the requirements set forth by major “pharmacopeias” to achieve the status of pharmaceutical raw material as suitability study of any bentonite before its use in manufacturing medicinal products is unavoidable [18,21].
Pharmacopeia contains detailed monographs on drug substances and dosage forms. Prior to pharmaceutical usage, raw material including bentonite must meet the United States Pharmacopeia specifications. For international markets, an excipient or drug must also fulfill the standards of British Pharmacopeia (BP), Japanese Pharmacopeia (JP) and European Pharmacopeia (EP) etc.

Keeping in view that, the basic requirements for bentonite to be used as pharmaceutical raw material are: mineralogical, microbiological and chemical inertness. The properties such as swelling power, gel formation, CEC, surface area and pore distribution, and particle size analysis are also important for particular uses. The mineralogical inertness dictate that in bentonite the crystalline silica must not be greater than 2% [22], as crystalline silica (both quartz and cristobolite) offers sufficient testimony of carcinogenicity in laboratory animals and limited evidence in human beings [23]. Also according to the International Agency for Research of Cancer (IARC), arsenic and arsenic containing compounds are deemed as human carcinogens [24]. Thus in bentonite samples, special attention must be drawn to Lead (Pb) and Arsenic (As), and must not be greater than pharmacopeia limit i.e. 40 and 5ppm for bentonite, 15 and 3ppm for purified bentonite respectively [25-27]. Microbiologically it is important that pharmaceutical raw bentonite must not be contaminated by Escherichia coli (E. coli) and the total viable aerobic count must not be greater than permitted range of major pharmacopoeias i.e. $10^3$ microorganisms per gram [27-28].
1.2 Problem Statement

The clay type, bentonite, is extensively used in Pharmaceutical industry both as an excipient and active principle in the forms of liquid, semisolid and solid either for topical or oral administration [10-15,29-31].

As natural material bentonites may vary in composition, and texture i.e. varies in toxic elements, non-clay minerals (crystalline silica) and microbial contents particularly Escherichia Coli, these all are harmful to the human health. Therefore, Prior to bentonite application in pharmaceutical sector as a adsorbent, opacifying agent, anticacking agent, binder, therapeutic agent, suspending agent, tablet and capsule diluents or disintegrant agent etc it is imperative that they must comply with some general features as high mineral, chemical and microbial purity. The physicochemical properties such as gel formation, swelling capacity, CEC, Surface area and porosity and particle size analysis are also important to be tested to investigate its specific use.

Before usage, the clay often has to be processed to increase the purity of the main phase, to improve the properties related to pharmaceutical application and to remove grit and non-swelling phases, such as quartz, feldspars etc. It is also important to mention that no bentonite can be equally well in the manufacturing of different medicinal product. Moreover, their specific function in any particular formulation depends on both their physical properties and chemical features. For example, its use as a suspending and tablet disintegrant agent mostly depend upon swelling and gelling properties while as a drug adsorbent and drug carrier in control drug release system depends upon its specific surface area, porous structure and cation exchange capacity value.
In Pakistan bentonite are found in abundance but none of them have neither been properly characterized nor investigated for pharmaceutical application. Therefore, the feasibility studies on the basis of major pharmacopeias are necessary and important to investigate its suitability for pharmaceutical use. To achieve this goal the knowledge of characteristics properties of bentonite related to pharmaceutical use and impurities from the characterization study of the raw material is first needed to adopt proper purification process/chemical treatments to make it suitable for use in pharmacy with improved properties. As expected, clay from all of pakistani sites may have different composition, properties and may required different processing technique for its pharmaceutical suitability, which is a wide range subject required huge fund and cannot be done in a single lab and human resources and not can be covered in a single PhD thesis. Therefore we have selected bentonite clay from three sites in Pakistan to limit the amount of work for this thesis.

In this study, the bentonite from Karak, Peshawar and Nowshera mines sites are collected and studied, characterized i.e. mineralogical, geochemical, microbiological and pharmacopeias suspension tests and then necessary processing technique were developed to purify the material for pharmaceutical use were carried out. These laboratories testing are essential to assess the potential application before suggestion can be made for further processing to make them suitable for pharmaceutical industry. This study will also provide the road map for processing using local bentonite in pharmaceutical industry.

No doubt the suitability study on local bentonite will generate an alternate income and the processing will convert the raw material into value added product for export and local use.
1.3 Aims and Objectives

The main aims of the proposed study are:

- To determine that the selected bentonite samples are swelling or non swelling bentonite (i.e. calcium bentonite or sodium bentonite).
- Mostly the local bentonites contain calcium montmorillonite; techniques will be developed to study the conversion through activation process from calcium bentonite to sodium bentonite.
- To study the suitability of local bentonite for use as a pharmaceutical product.
- In case of unsuitability of local raw bentonites, they will be purified with different methods to make it suitable for pharmaceutical use and to develop the research technique in order to produce particular quality bentonite for its subjective use.

1.4 Study Area

There are many different mines of bentonite in various part of Pakistan it was not possible for me to study the sample from all mine in the country. Therefore a case study of bentonite is carried out on samples collected from three different areas i.e. Karak, Nowshera and Peshawar district of Khyber Pakhtunkhwa (KPK), Pakistan. Further specify my study the locations of these mines are: Karak (Shagia) the mine is situated at the longitude of 071° 09.140'E and latitude of 33°04.572'N; At Nowshera the mine is situated in Dag Ismail Khel. This bentonite deposit occur at 071° 49.377'E longitude and 33° 51.591'N latitude. At Peshawar the bentonite was collected from Chandan Gahri which is located at the longitude of 71° 42' 12.84'E
and latitude of 33°49′0.66″ E. The mine photographs of the studied samples are shown in the fig. 1.1- 1.3.

**Fig.1. 1: Photograph showing the Karak (Shagia) mine.**

**Fig.1. 2: The Nowshera (Dag Ismail Khel) mine in Picture.**
1.5 **Scope of Study**

- Bentonite samples from three different sites Karak, Nowshera and Peshawar districts in raw form were used in this case study.
- Prior to the purification process, the raw samples were characterized using XRD, XRF, ICP-AES, SEM, Particle Size Analyzer, Surface Area Analyzer, CEC, Pharmacopeias Suspension Tests. This is important because to know that whether the local bentonites are suitable (i.e. fulfilling the basic requirement of pharmacopeia and having contents of crystalline silica within the permitted amount) or they failed to comply with required specification.
- After characterization and knowing the impurities in raw form further processing for purification, conversions and activations are adopted. In achieving the desired goal the following techniques were applied in order to
reduce the identified impurities and improve the required bentonite properties required for pharmaceutical application.

- Simple Sedimentation.
- Classical NaCl Treatment.
- Soda Activations followed by sedimentation.

After the due processing, the samples were tested as for pharmacopeias requirements.
CHAPTER-2

THEORY AND LITERATURE REVIEW

2.1 Clay and Clay Minerals

The use of clay and clay minerals in pharmaceuticals and cosmetics are very ancient. Clays are versatile and low cost naturally occurring material, which has been used since antiquity, characterized by a primitive economy, going through all historical eras of human civilization to our contemporary time. In present time we can find clay in natural, modified or synthetic form, as well as mixtures of clays with different origins and colors which may be used for various therapeutic purposes. The clay in raw form is inexpensive and abundant material worldwide. Though Clay is important, inexpensive and widely used industrial raw material but still there is some confusion in the meaning of clay and clay mineral.

The joint nomenclature committees (JNCs) of the Association Internationale pour l'Etude des Argiles (AIPEA) and the Clay Minerals Society (CMS) have recently pointed out that clay should not be used as a mineral term and an understandable distinction should be made between clay mineral and clay [2]. The JNCs defined “clay as a naturally occurring material composed primarily of fine-grained minerals, which generally show plastic behavior at appropriate water contents and hardness upon drying or firing” [2]. This definition of JNCs pointed that synthetic clays and clay-like materials are not defined as clay even though they may be fine grained, and display the characteristics of plasticity in water content and hardening upon drying.
According to Moore ‘clay’ is used as a mineral term, size term and a rock term [32]. Moore, further defined that, the term ‘clay’ can specify a rock, a sedimentary deposit, and the alteration (weathering) products of primary silicate minerals. In the literature the terms ‘fire clay’, ‘ball clay’, ‘bentonite’, ‘fuller’s earth’ and ‘bleaching earth’ have been used due to this one or other of these senses [33-34].

Although particle size is a key parameter in all definitions of clay but still there is no generally accepted upper limit [3]. Apart from it some disciplines and professions have conventionally set a maximum size of clay particles. For example, in sedimentology, geology and geo engineering the clay size limit is commonly restricted to 4µm in equivalent spherical diameter (e.s.d.) [7]. Weaver has also suggested 4µm is the upper limit of clay particle size [35]. In pedology, the ‘clay fraction’ refers to a class of materials whose particles are smaller than 2µm, while in colloid science the value of 1µm is generally accepted [3].

The term ‘clay mineral’ is also very not easy to define. “Clay mineral is a mineralogical term referring to a part of a family (the phyllosilicates) consisting of hydrated aluminosilicates with considerable amounts of Mg, K, Ca, Na and Fe and, occasionally, less common ions such as Ti, Mn, or Li and making up the fine-grained fraction of rocks, sediments, and soils” [18]. According to their different chemical composition, they can be categorized in just a few major groups: smectites, kaolin, micas, talcum, chlorites, vermiculites, fibrous and interstratified [1, 3].

According to JNCs Clay mineral is defined as “a phyllosilicate mineral and minerals which impart plasticity to clay and hard upon drying or firing” [2].

Since neither origin of the material and nor grain size feature is the part of the definition, clay mineral may be synthetic. Therefore, phyllosilicates of any size, such
as macroscopic mica, chlorite and vermiculite may be considered as clay minerals. It was further proposed that if any non-phyllosilicate minerals impart plasticity to clay and hardening upon drying or firing then they will also be regarded as clay minerals [3].

The distinction between clay mineral and clay must always be noted, as these are two different thing clearly explained. However, the term ‘clay’ is often uses for ‘clay mineral’ in the literature because the former is shorter and manageable.

The most commonly used clays in industry are: (i) bentonite with montmorillonite as the main clay mineral constituent, (ii) palygorskite and sepiolite, (iii) kaolins containing kaolinite, and (iv) ‘common clays’ which often contain illite/smectite mixed-layer minerals ([1,3]).

2.2 Bentonite Clay

Fundamental of bentonites in terms of its basic introduction, mineralogy and industrially attractive properties are discussed in this sub-section.

2.2.1 Introduction and Origin

Bentonite is a rock or a clay base important industrial material. It is therefore, bentonite is a mixture of minerals. The term bentonite was named after Fort Benton (Wyoming, USA), the locality where this type of clay was first discovered by American geologist Knight in 1898. Early in the 20th century, many geologists recognized that bentonite originated from transported volcanic materials. Ross and Shannon [36] defined bentonite which is widely quoted and generally accepted by geologist as follows:
“Bentonite is a rock composed essentially of a crystalline clay-like mineral, usually the mineral montmorillonite, but less often, beidellite, formed by the devitrification and the accompanying chemical alteration of a glassy igneous material, usually a tuff or volcanic ash”

The above definition for bentonite is origin based and is restricted to volcanic ash, tuff, or glass parent material. Therefore, deposit consisting of smectite having uncertain origin or parent material cannot properly be called bentonite.

Perhaps the best definition of bentonite is one proposed by Grim and Guven [6] which differentiates the geological and industrial usage of the term and overcome the difficulty in assigning a name to smectite that formed from igneous rock other than volcanic or sedimentary or other uncertain origin. According to their definition “bentonite is any clay which dominantly comprised of smectite mineral (montmorillonite group in some usage) regardless of the mode of origin and occurrence”.

Montmorillonite is named after a Montmorillon (France), the city where it was first discovered in 1874 [37]. In the older literature the term “montmorillonite” was frequently used both for related clay minerals and for specific member of that group [38]. Presently the word smectite is the group name and montmorillonite is restricted as a mineral name belonging to that group [39]. Smectite is the group name for several hydrated sodium, calcium, magnesium, iron, and lithium aluminum silicates. The individual mineral names in the group are sodium montmorillonite, calcium montmorillonite, saponite (Mg), nontronite (Fe), and hectorite (Li). Generally smectite has a particle range between 0.1µm to 2 µm and average particle size is 0.5 µm [1, 40]. Because of their ability to swell they possess a variable dimension along
c-axis. It ranges generally from 12-15Å in air, depending on the type of interlayer cations, layer charge and relative humidity. Saturation with ethylene glycol, smectite swell to about 17Å, regardless of these factors and therefore these treatments are commonly used for smectite identification.

A rock which contains Ca$^{2+}$-montmorillonite or Na$^+$-montmorillonite as major clay minerals is called calcium bentonite or sodium bentonite respectively [6]. Commercial Bentonite contains either Ca$^{2+}$-montmorillonite or Na$^+$-montmorillonite and lower amount of other minerals [6, 41].

### 2.2.2 Bentonite Clay Mineralogy

As previously discussed that bentonite is a rock term predominantly comprised of smectite group of clay minerals.

Fig. 2. 1: Diagrammatic representation of (a) Single tetrahedral unit and (b) The sheet of tetrahedral units

- Oxygen
- Silicons
The basic structure of smectite clay minerals consist of an octahedral sheet sandwiched between two tetrahedral sheets [1, 3]. This is called 2:1 Tetrahedral-Octahedral-Tetrahedral (TOT) layer structure. The thickness of the individual TOT layer of smectite is about 1nm [42]. The basic building blocks of these tetrahedral and octahedral sheets are tetrahedra [SiO₄] (fig 2.1) and octahedra [M (O, OH)₆] (M= Al³⁺, Mg²⁺ Fe²⁺ or Fe³⁺ etc) unit respectively shown in fig 2.1 and fig 2.2.

![Diagram of octahedral unit and sheet of octahedral units](image)

**Fig.2. 2: Diagrammatic representation of (a) Single octahedral unit and (b) The sheet of octahedral units**
In this 2:1 layer structure, the unit cell includes six octahedral sites and eight tetrahedral sites. Structures with four out of the six octahedral sites occupied (i.e. dominantly occupied by trivalent cations) are known as di-octahedral smectite. If all the six octahedra are occupied (i.e. dominantly occupied by divalent cations), the smectite is referred to as tri-octahedral. Fig. 2.3 shows the di-octahedral and tri-octahedral smectites.

![Diagram of Smectite structure]

**Fig.2. 3: The individual minerals names in di-octahedral and tri-octahedral smectite.**

The theoretical formula for smectite clay minerals per unit cell in the absence of isomorphic substitutions is $\text{(OH)}_4 \text{Si}_8 \text{Al}_4 \text{O}_{20} \cdot n(\text{interlayer}) \text{H}_2\text{O}$ and the theoretical composition without the interlayer material is 66.7% SiO$_2$, 28.3% Al$_2$O$_3$, and 5% H$_2$O [1]. The theoretical charge distribution in the smectite layer without considering

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substitutions in the structure is as shown in the fig 2.5. However smectite minerals are produced due to full or partial replacement of Al$^{3+}$ in the octahedral sheet and partial replacement of Si$^{4+}$ in the tetrahedral sheet [5, 43]. The substitution of Si$^{4+}$ in the tetrahedral sheet is commonly limited to only 15% by mainly Al$^{3+}$ ions and of Al$^{3+}$ in the octahedral sheets are generally by various type of cations such as Mg$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Zn$^{2+}$, Li$^+$ etc [1,5]. This substitution causes the net negative electric charge in the 2:1 (TOT) layers. Bargaya et al. [3] pointed out that many analyses have shown that this net charge deficiency is ranged from 0.4 –1.2 electronic charge of per unit cell. This electric charge deficiency is balanced by the exchangeable cations such as Na$^+$, Ca$^{2+}$, K$^+$ and Mg$^{2+}$ located between the layers and surrounding the edges[44].

The di-octhedral smectite mineral montmorillonite is the most commonly found component in bentonite. The crystalline structure of montmorillonite is shown in fig. 2.4.

![Diagrammatic sketch of the structure of montmorillonite.](image-url)
In montmorillonite case the net charge deficiency results from substitution of one Mg$^{2+}$ occur in every sixth Al$^{3+}$ in octahedral sheet with no substitution in the tetrahedral sheet. Fig.2.5 shows the charge distribution in a montmorillonite unit cell, where the calculated electric charges are in the term of charge on electron. This substitution results charge deficiency of about 0.66 e/ unit cell. The chemical formula for a unit cell of montmorillonite with Na$^+$ as an interlayer cation is written as $[\text{Si}_8(\text{Al}_{3.34} \text{Mg}_{0.66}) \text{O}_{20}(\text{OH})_4]. \text{Na}_{0.66}]$. The other exchangeable cation commonly found within the interlayer spaces are Ca$^{2+}$, K$^+$ and Mg$^{+2}$.

![Fig.2. 5: Charge distribution in 2:1 layer of montmorillonite [45].](image)

It is reported that the structural formula is often written on the basis of the half unit cell content i.e. it is based on three octahedral sites [3].
The dioctahedral smectite have the general formula.

\[
(M^+_{x+y} \times nH_2O)(R_{2-x-y}^{3+}R_y^{2+}\times Si_{4-x-y}^{4+}Al_x^{3+})O_{10}(OH)_2
\]

And formula for trioctahedral smectites is:

\[
(M^+_{x} \times nH_2O)(R_{2-x-y}^{3+}R_y^{2+}\times Si_{4-x-y}^{4+}Al_x^{3+})O_{10}(OH)_2
\]

Where \( M^+ \) represents the exchangeable cation; \( R^{3+} \) and \( R^{2+} \) refer to generic trivalent and divalent octahedral cation respectively; \( x \) and \( y \) represent the layer charge resulting from substitutions in tetrahedral and octahedral sites respectively [3].

**Table 2.1: Chemical formulas of smectite group clay minerals.**

<table>
<thead>
<tr>
<th>Smectite</th>
<th>Chemical formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montmorillonite</td>
<td>((M^+<em>y \times nH_2O)(Al</em>{2-y}^{3+}Mg_y^{2+}\times Si_{2}^{4+}Al_{y}^{3+})O_{10}(OH)_2)</td>
</tr>
<tr>
<td>Beidellite</td>
<td>((M^+<em>x \times nH_2O)Al</em>{y}^{3+}\times Si_{4-x-y}^{4+}Al_x^{3+})O_{10}(OH)_2)</td>
</tr>
<tr>
<td>Nontronite</td>
<td>((M^+<em>x \times nH_2O)Fe</em>{2-y}^{3+}\times Si_{4-x-y}^{4+}Al_x^{3+})O_{10}(OH)_2)</td>
</tr>
<tr>
<td>Volkonsoite</td>
<td>((M^+<em>x \times nH_2O)(Cr</em>{2-y}^{3+}\times Si_{4-x-y}^{4+}Al_x^{3+})O_{10}(OH)_2)</td>
</tr>
<tr>
<td>Saponite</td>
<td>((M^+<em>x \times nH_2O)(Mg</em>{2-y}^{2+}\times Si_{4-x-y}^{4+}Al_x^{3+})O_{10}(OH)_2)</td>
</tr>
<tr>
<td>Hectorite</td>
<td>((M^+<em>y \times nH_2O)(Mg</em>{2+y}^{2+}\times Li_{y}^{3+}\times Si_{4-x-y}^{4+})O_{10}(OH)_2)</td>
</tr>
<tr>
<td>Sauconite</td>
<td>((M^+<em>x \times nH_2O)(Zn</em>{3-y}^{2+}\times Si_{4-x-y}^{4+}Al_x^{3+})O_{10}(OH)_2)</td>
</tr>
</tbody>
</table>

The general composition of the most important end-members of smectite derived from the above two general formulas on the basis of possible isomorphic substitution and interlayer cations are shown in Table 2.1.
Exchangeable cation such is $Ca^{2+}$, $Na^{+}$ and $Mg^{2+}$ occur between the silicates layers associated with water molecules. These water molecules within the interlayer in smectite are called low temperature water which can be driven off easily by heating it from 100-150°C [5]. Low [46] pointed out that the water exists between montmorillonite layers is in physical state different from liquid water. It has also been studied that the water molecule within interlayer are structurally oriented to form an ice-like structure [47]. Johnson et al. [48] recently investigated that thickness of these water molecules is related to interlayer exchangeable cations. In case of Na+, the water layer is limited to 2.5 Å which is one water layer and $Ca^{2+}$ or $Mg^{2+}$ case the layer is about 4.2-4.5 Å thick, which are two water layers. A $Na^+$-montmorillonite has a spacing layer of about 12.5 Å and a $Ca^{2+}$-montmorillonite case the layer has a spacing of about 14.2-14.5 Å.

The smectite clay minerals vary in color from tan to brown to brownish green or blue green and are rarely white [1]. Some important properties of smectite that make them valuable in industrial sector are: high CEC, high surface area, high absorption capacity, high swelling capacity, high viscosity and small particle size.

2.2.3 Industrially Attractive Properties of Bentonite

Bentonite is a natural material varies in composition and texture. Hence they are not perform equally well for specific industry. Bentonites are mainly composed of a Montmorillonite with minor contents of impurities that give it a unique “fingerprint. The more montmorillonite in the bentonite ore, the better it will perform as an industrial raw material [1]. The other clay, non clay mineral, heavy and trace element, and pathogenic microorganism that might be present in bentonite, reduce its net
drawing power. For example, the mineral kaolinite, mica, gypsum, calcite, crystalline silica (quartz and cristobolite), the toxic element especially arsenic and lead, the photogenic bacteria i.e. Escherichia Coli and salmonella species and other organic compound might be present in bentonite [1,6,18] which dilutes its potential use in industries especially in pharmaceutical sector [1,3,18,22]. Apart from its purity, bentonite ability to industrial use comes from several properties but the most important are described below:

i. **Cation Exchange Capacity**

The cation exchange capacity (CEC) is one of the basic properties of clay minerals. Cations are attracted and held in between the sheets and on the edges of particles in order to maintain the electro-neutrality of particle charges. CEC is defined as “the quantity of cations reversibly adsorbed by clay particles”, expressed as milliequivalents per 100 grams (meq/100g) of dry clay [49]. The total CEC consists of two parts namely the exchange capacity of the interlayer and that of the edges. The interlayer exchange capacity comes from isomorphic substitutions of aluminium by magnesium at octahedral and /or silicon by aluminium at tetrahedral leads to the net negative charge, the cations then balances this ionic deficiency. This part is independent of pH value, so gives almost constant value.

The CEC results from the edge are strongly pH dependent. At pH 7 about 20% of the CEC of smectite is located at the edges [50-51]. In the acid pH range, the edges are positively charged; thus the edges do not contribute to the measured CEC. While in the basic pH range, the edges are negatively charged; therefore in this pH range all edges contribute to the CEC. The CEC values are expressed in unit recommended by
IUPAC known as centimole of positive charge per kilogram of dry clay sample (cmol(+)/kg), which is numerically equal to the traditional unit called milliequivalents per hundred gram of clay (meq/100g).

Smectite have high CEC, which is in the range of 40-150 meq/100g. This high CEC of smectite is due to the high isomorphic substitution occurring within the octahedral and tetrahedral layers [1]. Sodium montmorillonite has a CEC which is generally between 80 and 130 meq/100g. On the other hand, Ca\(^{2+}\)-montmorillonite has a CEC normally ranges from 40-70 meq/100g. Among the clay minerals, the bentonites enjoy wide applications in the pharmaceutical industry because of their high swelling and high CEC [12, 30, 52]. It is due to their high CEC that smectite can interact with certain drugs affecting their bioavailability. Nevertheless, this interaction could be advantageous in the formulation of controlled release systems, which is one of the most attention-grabbing fields of clay applications at present [14, 31]. The variation in montmorillonite interlayer cations and CEC are resulting changes in drug loading ability or adsorption capacity [53].

ii. Swelling

The most important property of smectite and corresponding bentonite is the uptake of water in the interlayer space and the resulting swelling of the mineral. Swelling is defined as the change in physical state of clay from an anhydrous solid to gel [54-55]. It is due to this property that bentonite can be used as a disintegrate agent for tablets formulation, for preparation of oral and topical suspension [18, 56-58]. There are two stages of swelling: one is the crystalline and the other is osmotic swelling. In former case the smectite can take water gradually from one to four layers
[59-60], controlled by balancing between strong electrostatic and hydration repulsive forces [46, 61-64]. While in osmotic swelling complete dissociation of the smectite layers occur [60, 65], which is generally based on Defuse Double Layer Theory [66-69]. The increase in basal spacing by swelling depends on the bentonite mineralogy, types of exchangeable cation, magnitude of the layer charge and hydration of exchangeable cations [65,70]. Usually sodium bentonite develop higher swelling than calcium ones, therefore for specific purposes the purification and conversion techniques are applied to improve the properties especially swelling of poor bentonite [40]. According to Japanese pharmacopoeia [71] the required swelling property of bentonite for pharmaceutical use is ≥20ml, while specified by European pharmacopeia [27] and US pharmacopeia [25] are ≥22 ml and ≥24ml respectively.

iii. Surface Area and Porosity

Bentonites are porous in nature. The high contents of montmorillonite contained in the bentonite are the main source of porosity. In a solid, the voids located among and within particles are known as pore. The pores can be cylindrical, parallel-sided silt, cavity and ink-bottle of different sizes [70]. The IUPAC classified the pores by their internal pore width [72]. The diameter of cylindrical pore and the distance between opposite walls in case of silt pore can be taken as the internal width. The pores with internal widths greater than 50 nm, between 2 nm and 50 nm and smaller than 2 nm are called macropores, mesopores and micropores respectively [72-74]. The macropores lie among the particle while mesopores and micropores lie within the particle [9]. These three types of pores are often found in bentonite. The
physicochemical properties of bentonite especially adsorption depends extensively on mesopores and micropores [9].

The pores area of the inner and outer walls in one gram of solid is defined as specific surface area [75]. Surface area approximately corresponds to the walls of micropores and mesopores. The contribution of the macropore surface to the total surface area is negligible [75]. The pores volume in one gram of solid is taken as specific pore volume. A number of methods have been investigated to determine pore-size distribution, surface area and pore volume. They include mercury porosimetry, $N_2$ adsorption-desorption and thermoporometry [72, 76].

2.3 Bentonite from Pakistan

In Pakistan, workable deposits of bentonite occur at different places in Punjab, Sindh, Khyber Pakhtunkhwa provinces and Azad Kashmir.

i. Punjab

Alauddin et al. [77] have reported bentonite from the Campbellpur (Attock). This clay is white in colour and occurs near the village of Dherikot, about 10 km south-west of Attock City. This deposit is in the vicinity of the left bank of Haro River, a tributary of the Indus. The area is considered to be a part of the Kala Chitta hills, the outer ranges of the Himalayas. These clays mainly composed of montmorillonite with small amount of feldspar and quartz. Yousaf et al. [78] have described a valuable bed of bentonite in Dera Ghazi Khan. At Rohtas and Ganda in the Jhelum district reasonable big deposits of bentonite have been reported. These beds have a maximum thickness of about 4 ft [6]. Other deposit of bentonite has been found in Attock, Chakwal, Mianwali, Khushab districts [6,79-80].
ii. **Khyber Pakhtunkhwa (KPK)**

KPK is also rich with several deposits of bentonite. The big deposit of bentonite has been found at Shagia, a village of district Karak. This deposit spread over an area of about 18 km². The 36 million tons of bentonite have been investigated in this area [81]. This bentonite is grey in colour composed of montmorillonite as a major, illite and kaolinite as minor clay minerals and quartz as a non clay mineral. The occurrence of volcanic ash deposits of bentonite at Shamshatu area, Cherat hills, about 25 km to the south-east of Peshawar have been investigated by the Geological Survey of Pakistan. This bentonite mainly composed of Ca-montmorillonite with subordinate of illite and quartz [82]. A reasonable big mine of bentonite have been found in Nowshera district. This deposit Occur in the region about one kilometer towards east of the Dag Ismail Khel and about five kilometer from Aza Khel toward west [83]. The bentonite, originating from Jehangira occurs at 33° 59' 56" latitude and 72° 12' 47" longitude in the survey of Pakistan topographic sheet [81,84], mainly composed of Ca²⁺-montmorillonite. Recently it has been concluded that jahangira bentonite can be used to replace cement up to 30% to produce concrete with sufficient compressive strength [85].

iii. **Sindh**

A thick bed of bentonite, runs about 12 km along the base of a scrap of Eocene rocks formation in the Shadi Shahid hills, occurs about 5 km east of Khairpur. The main minerals found in this bentonite are montmorillonite and small amount of illite, kaolinite, gypsum and quartz [86].
iv. Azad Kashmir

The bentonite deposits at Dudial and Mirpore in Azad Kashmir have been investigated. Bentonite deposits of Dudial area are located between Pir Mekal-Sadiqabad and Kathar-Meru gala villages. The Dudial town is located at a distance of about 75 km from Mirpur-Dongali Rawalpindi road [87]. The other deposit of bentonite also have been found at Bimbhar, Mawa, Kenali, pothi and Karoth in azad Kashmir areas [6,88].

Pakistani scientists states that the bentonite occurring in Pakistan are formed from volcanic ash which was deposition in shallow estuarine and lagoonal waters during the tertiary period [82,88,89]. Deposition to have taken place only once in some area and appear several in others. This view is supported by the evidence of wide spread volcanism in the Himalayas during the tertiary period [89]. The Pakistani bentonite is gray, bluesh, off white, yellowish, white and dark brown colors. The bentonites found in Pakistan are almost calcium bentonite and no sodium bentonite mines have been yet explored. The associated mineral in our local bentonite deposit are quartz, feldspar, biotite, carbonate and gypsum.

In Pakistan, presently most of the bentonite deposits are under regular mining and available in our local market. Although bentonites are used in many industries but Pakistani bentonite has not been evaluated for pharmaceutical purposes.

2.4 Uses of Bentonite

Bentonite is the most important industrially applied clay because of its main montmorillonite component which gives it a very high sorbent ability and swelling capacity [1, 90-91]. Sodium Bentonite clay is particularly of great interest due to its
formation of a gel like structure at relatively low clay concentration and high swelling capacity [92]. For specific industrial use the bentonite properties can be controlled and the conversion of bentonite from one form to another can be possibly obtained by chemical treatment and purification process [93]. The uses of bentonite in pharmaceutical industry are discussed in detail in the subsection, another uses are briefly mentioned below.

Bentonite are used extensively as a bonding material in the preparation of molding sand (mixture of sand and bentonite) for the production of shaping metals in the casting process [1,3,94-95]. Bentonite provides the plasticity to sand- clay mixture so that it can be molded around the pattern. The important properties of bentonite yield sand moulds with good compact-ability, flow-ability, and thermal stability for the production of high quality casting. Another important and conventional use of bentonite is as a mud constituent for oil well drilling [1]. The role of the bentonite in drilling is mainly to seal the borehole walls, to lubricate the cutting head and to remove cutting from the drill hole. High viscosity and gel formation properties are required in order to prevent the cutting from the settling to the bottom and remove it from the drill hole. Mostly sodium bentonites are used in drilling fluid because of its high viscosity, high swelling and gelling properties.

Bentonites are used as a binding agent in the production of iron ore pellets [96]. Finely pulverized iron ore concentrates are converted into spherical pellet of about 2.5cm in diameter for easiness of handling and shipping and to produce a superior furnace feed. Sodium bentonite is preferably used for pelletizing because of its best dry strength and its low percentage is enough to bind the pellet. Sodium bentonites are also important to paper making where it is used to prevent agglomeration of pitches,
waxes and resinous material so that it will not stick to machine, wires, screen and press roll etc. Hence not causes any defect and holes in the paper but improve the quality [1, 3, 6].

Bentonite is also useful in the deinking process for paper recycling [1]. In the de-inking process the ink pigment are first released by heating the recycled paper in a caustic soda solution and then added sodium bentonite to absorb the ink pigment. The bentonite are then removed by washing procedure. In addition, acid activated bentonite is used as the active component in the manufacturing of carbonless copy paper. Bentonite is used as constituent in cat litter because of its high absorbent quality and swelling power [6, 96-97]. In this case when the feline waste hits the granular product of bentonites, they swell and form a hard clump which can easily be removed from the litter box and leaving the remaining product safe for further use.

Sodium bentonites are also famous for water impedance for their high swelling power. Bentonites swell and fill the voids and pores in the material into which they are incorporated, preventing liquid from moving through the barrier. Bentonites are commonly used in dams, ponds and impound to prevent seepage of water. They are also used to prevent water from entering basements of homes. In landfills and toxic waste dumps sodium bentonite are used to prevent liquid from entering and exiting [97].

Bentonite in acid activated form are used to refine and bleach palm oil, sunflowers, rapeseeds, animal fat, soybean, corn oils because of its high adsorptive property [98-99]. In drinks such as wine, bear, mineral water and in products like honey or sugar bentonites are used as clarifying agent. The more refining uses of
bentonite are as carrier for pesticides and fertilizers, purification of waste water and animal feed binder [1, 6, 40].

Bentonite is also used in Portland cement and mortars due to its viscosity and plasticity. The additions of small amount of sodium bentonite in cement improve the workability and impermeability. It has also been reported that bentonite is acceptable pozzolan in cement [100]. In ceramic industry Bentonite is not used as a major component but to improve the plasticity and dry strength small percentage of sodium or calcium bentonite is added [101]. Sometime bentonite is added to porcelain to lower the firing temperature and as a suspending agent. sodium bentonite in small amount can also be added to glazes as a suspending agent [1]. Bentonites with sodium montmorillonite are also playing an important role in paints where they function as a thickening, emulsifying and suspending agent. The adsorption properties of bentonite are appreciated for dyeing cloths and lacquers for paints and wallpapers. Bentonites are extensively used in cosmetic and pharmaceutical industry as an excipient and active ingredients [14,18], as described in more detail in the following section.

2.4.1 Pharmaceutical Uses of Bentonite

A medicinal product is a substance or combination of substances administered to humans in order to treat or prevent illness, carry out a diagnosis or restore, correct or modify disturbed physiological functions. Bentonite is used as medicinal product in industry as both excipients and active principle in the forms of liquid (suspensions, emulsions), semisolid (creams, ointments) and solid (capsules, tablets and powders) either Pharmaceutical for topical or oral administration [10-15]. The usefulness of the
clay minerals in pharmaceutical application are due to different properties such as high adsorption ability, interlayer reactions, high surface area, chemical inertness, high cation exchange capacity, low or null toxicity for the patient and low price [17,52].

2.4.1.1 Use as an Active Substance

i. Oral Administration

Bentonite administered orally to the patient may act as antacids, gastrointestinal protectors, antidiarrhoeics, and osmotic oral laxatives.

Antidiarrhoeal Uses: Diarrhoea is either an acute or chronic pathological condition, may be caused by defective intestinal absorption, bacterial infection, allergy, intoxication etc. In Diarrhea the fluidity and frequency of evacuation of the feces increased. Clays including bentonite are widely used as anti-diarrhoeics for its high specific surface, sorption capacity, and non-toxic to patient. Smectite and the corresponding bentonite are used in the treatment of infectious diarrhea as they reduce the fluidity and frequency of evacuation of the feces by mechanisms including absorption of water and electrolytes in the intestine, protection of the luminal surface against pathogenic bacteria and decrease of mucolysis caused by bacteria [102-103]. In addition, some authors have described the use of bentonite in the cure of acute diarrhoea, although no experimental evidence supports this statement [104-106]. Prior to its usage, smectite are commonly “activated” by acid or heating in order to enhance their antidiarrhoeaic efficiency [8, 107-108]. The long-term use of clay minerals is not recommended as antidiarrheas because of the risk of renal silica calculi (kidney stone) formation [109-110].
**Gastrointestinal Protectors:** In patients suffering from peptic ulcer, the thickness of the mucus layer formed on the gastro-duodenal mucous membrane [111], act as a physical barrier preventing direct contact between the gastric enzymes and the cells of the mucous membrane [112], decreases while the mucolytic activity of the gastric enzyme levels increase [113-114]. Clay (e.g. bentonite) used as effective gastrointestinal protectors because of its high sorption capacity, large specific surface and non-toxic to organisms [115-116]. These clay minerals reduce irritation and gastric secretion; take up gases, bacteria, toxins, and even viruses while adhering to the gastric and intestinal mucous membrane [117]. The mode of action of clay mineral involves stability of the gastric mucus, increasing the viscosity and decreasing the degradation of glycoprotein in the mucus [14-117]. The use of sodium smectite as osmotic laxative was also illustrated by Carretero (2002)[12], though no experimental evidence support this statement.

**Antacids:** Antacids are drugs which are administrated orally to the patient in the form of tablets, suspensions, or powders to reduce the gastric acidity arising from the excess production of hydrochloric acid in the stomach [30]. The clay minerals can act as antacids by reducing the gastric acidity through the adsorption mechanism of H+ ions (protons) to the surface. The main reaction products are silica gel, and interlayer cations in the case of smectite [30]. The continual use of clay minerals is not recommended as antacids, because of the formation of renal silica calculi [109-110]. The mixture of different antacids has the extra advantage of prolonging the therapeutic effect of the pharmaceutical preparation.
ii. **Topical Administration**

Because of high sorption capacity, smectite can also be administered to the patient topically (to the exterior or on a limited portion of the body) as dermatological protectors to protect the skin against external agents and, occasionally, taking up agents exuded by the skin itself [30,118]. They are generally in the form of ointment, creams and powders. Smectites are applied as dermatological protectors to adhere to skin, forming a film to mechanically protect the skin against external physical or chemical substances and recommended for inflammatory process such as acne, boils, ulcers [14, 17, 30]. Bentonites are also used in antiseptic purposes [14]. The amalgamation of Bentonite with some other clay mineral can be employed as dressing for the treatment of skin injuries [14, 119].

2.4.1.2 **Use as Excipients**

Bentonites with higher amount of smectite are widely used as excipients in pharmaceutical formulation. The word excipient means, an agent to facilitate the pharmaceutical formulation and to improve the organoleptic properties i.e. smell, taste and colour, physical and chemical properties (e.g. viscosity), facilitate liberation of the active ingredient within the organism (carrier-releasers, disintegrates), and their elaboration (lubricants, binders, diluents agents). The different roles of bentonite as excipients in pharmaceutical formulation are summarized below.

**Disintegrants, Binders and Diluents:**

Bentonite serves as diluents, tablet disintegrants and binder agent. The using of bentonite as disintegrant agent play an important role in liberating the active ingredients administered to the patient in the form of tablets, because of its high swelling capacity and readily decomposition in the acidic medium of stomach. It is
investigated that bentonite disintegrate efficiency is more effective comparable to other topical disintegrants such as cellulose derivative [120]. In addition, smectite also facilitates the compaction of tablets. The uses of bentonite as tablet and capsule disintegrant, and tablet binder have been studied by several authors [121-123]. Bentonites can also be used as diluents in pharmaceutical preparations because of their plastic nature at moisture content and are non-toxic to the patient. Smectite are also useful diluents for cosmetics. Bentonites with high contents of smectite are very useful as stabilizers for their thixotropic properties [124].

**Suspending, Emulsifying, Anticaking Agents and Flavour Correctors:**

The pharmaceutical preparations in the form of liquid for oral or topical administration, there is a need of an agents to prevent segregation of the components, and avoid formation of sediment that may be not easy to re-suspend suspensions, syrups and gels etc. Bentonites are widely used as suspending, emulsifying, anticaking and stabilizing agents in the formulation of suspension, ointment, cream and gel because of their thixotropic and colloidal characteristics [14, 92,125-126].In the case of smectite, the particles can interact in an ‘face-to face’ and ‘edge-to-face’ fashion to form a rigid system [127-128].Bentonites are also wildly used as flavour correctors in pharmaceutical preparations to fix the unpleasant test of orally administrated active ingredient [14, 30].

**2.4.1.3 Carriers and Releasers of Active Ingredients**

Smectite and the corresponding bentonite are well suited to act as drug carriers and releasers, because of their large specific surface area and high cation exchange capacity [129]. McGinity and Lach have been examined the Amphetamine sulfate-montmorillonite complexes as a method of controlling the drug release [130].
drug concentrations were tracked by urinary recovery studies and found montmorillonite effective in prolong drug release system. Shrivastava et al. showed that at acidic pH, the Metronidazola- montmorillonite complex and their corresponding physical mixture were effective in inhibiting drug release [131]. In order to obtain continual action in the administration of oral antihistamines, the interaction between montmorillonite and chlorpheniramine maleate was studied [132]. Lin et al. have reported the use of montmorillonite as a slow release vehicle of a 5-fluorouracil drug against colon cancer suitable for oral administration [52]. Some authors have also reported that acid activated bentonites are more effective than natural ones in extended drug release formulation [133-135]. Pharmaceutical preparations, using bentonites as a carrier and releasers of active ingredients, can generally be administered orally to the patient.

2.4.1.4 Bentonite in Cosmetic Products

Cosmetic products such as Creams, powders, emulsions are applied to external parts of the body in order to beautify or change physical appearance, or care for the physico-chemical conditions of skin. The opaque material, bentonite with a high sorption capacity are commonly used as active principles in cosmetic formulations as creams, powders, emulsions in order to remove shine, give opacity, and cover blemishes [12, 30]. In addition, adhering to skin and forming a protective film, bentonite can absorb grease and toxins.

2.5 Pharmaceutical Specification of Bentonite

Bentonite is natural material which may vary in composition and texture, therefore before its use in pharmaceutical sector they must comply with the guidelines set forth by the major pharmacopoeias [25-27, 71, 136], shown in the table 2.2.
Table 2.2: Pharmaceutical specification of bentonite as indicated in major pharmacopoeias.

<table>
<thead>
<tr>
<th>Pharmacoeial specification</th>
<th>LIMITATION</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>USP</td>
<td>EP</td>
<td>BP</td>
<td>JP</td>
</tr>
<tr>
<td>Chemical limitation</td>
<td>Pb</td>
<td>≤ 40ppm</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>As</td>
<td>≤ 5ppm</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Technical properties</td>
<td>PH</td>
<td>9.5-10.5</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>Swelling power</td>
<td>≥24ml</td>
<td>≥22ml</td>
<td>≥22ml</td>
</tr>
<tr>
<td></td>
<td>Sedimentation volume</td>
<td>≤2ml</td>
<td>≤2ml</td>
<td>≤2ml</td>
</tr>
<tr>
<td>Microbial Tests (cfu/g)</td>
<td>Total Microbial count</td>
<td>≤1000</td>
<td>≤1000</td>
<td>≤1000</td>
</tr>
<tr>
<td></td>
<td>E. Coli</td>
<td>Absent</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

Specific attention must be drawn towards the Crystalline silica (both quartz and cristobalite) as it is found more often than not in bentonite and should be avoided above 2% proposed for pharmaceutical use [22], as it offers sufficient evidence of carcinogenicity in laboratory animals and to some extent in humans [23].

Tateo et al [137] observed that toxic trace elements such as As, Cd, Pb, Te, Hg, Tl, Sb and Se exist in low concentration after digestion, but pinpointed that ingesting clays without knowledge of their composition may be dangerous. Due to side effects of the toxic trace elements, the use of clay products in health must be legally bound [138-139]. Due attention must be paid to the amount of Pb and As subsisting in the Bentonite. According to the International Agency for Research of Cancer (IARC), arsenic and arsenic containing compounds are deemed as human carcinogens [24].
CHAPTER-3

MATERIAL AND EXPERIMENTAL TECHNIQUES

3.1 Collection of Samples

The raw bentonite Samples used in this study were obtained from three different sites in Khyber Pakhtunkhawa province of Pakistan. These are located in Shagai district Karak, Dag Ismail Khel district Nowshera and Chandan Garhi district Peshawar. Bentonites from these sites are classified as Karak, Nowshera and Peshawar bentonite respectively after the name of districts in which the mines are situated.

The Karak deposits located at 33°04.572′ N latitude and 071°09.140′ E longitude, spread over an area of about 18 square kilometer. The existence of 36million tons of bentonite has been estimated in this deposit [81]. The bentonite found in Karak is mainly containing Smectite, Illite, and Kaolinite clay minerals [140]. The color of this clay is gray type and the thickness of the beds over which the deposits occur varies from 3 to 5 meter.

The Nowshera bentonite mine located in the region about two kilometers away from Dag Ismail Khel in Nowshera district towards north east. This deposit is situated
at the longitude of 071°49.377E and latitude of 33°51.591N. This sedimentary deposit occurs beneath the earth surface about 3-4 meters. The bentonite obtained from this deposit is yellowish in color.

Fig. 3.1: Location map of the three studied samples.

Peshawar deposit Occurs in the region near the village ChandanGarhi about 500 meters towards south at 33°049.011′N latitude and 071°42.214′E longitude. The color of this clay is off-white and the thickness of the beds varies from 2 to 3 meter. The combined location map of all the three Pakistani mining sites is shown in fig. 2.2.

3.1.1 Sample Preparation

The samples in rock form were collected from the above mentioned sites and prepared powder bentonite for characterization study. The wet sieving method was
adopted by dispersed each sample in distilled water for 48h, stirred occasionally by mechanical stirrer and sieved by using mesh No-325 sieve (ASTM). The sieved clay suspension was then dried at 60°C. The dried samples were further ground, sieved through mesh No-120 and placed in a dry controlled environment for experimental use and designated as “raw bentonites”.

3.2 Purification of Raw Bentonite

In order to separate the main clay particles (particle size-fractions ≤ 2 μm) from associated minerals such as crystalline Silica (quartz, cristobolite), feldspar etc, and to improve its related properties to pharmaceutical application, three different purification methods (i.e. Simple Sedimentation using Sodium hexametaphosphate (Na (PO$_3$)$_6$) as a dispersing agent, Classical NaCl Treatment Method and Soda Activation Method followed by sedimentation process) were applied on the raw bentonites previously leached for 48 hours.

3.2.1 Method-I: Simple Sedimentation

Five grams of each raw bentonite sample was suspended in one litre(1 L) of de-ionized water with about 0.25 g of dispersant agent (Na (PO$_3$)$_6$) and stirred magnetically for 1hr. By letting the suspension stand at constant temperature and decant the supernatant of clay suspension above certain depth after the specific time at 25°C estimated from the following relation, based on stoke law, assuming the spherical shape of the solid particle.

$$t = \frac{18\eta h}{(\rho - \sigma)gd^2}$$
Where

\( \rho \): density of the spherical material = \( 2.65 \times 10^6 \) g/m\(^3\)

\( \sigma \): density of liquid = \( 0.997 \times 10^6 \) g/m\(^3\)

\( \eta \): viscosity of the fluid = 0.89 g/ms

\( h \): depth from the surface of suspension in cylinder = 0.1 m

\( g \): Gravitational acceleration = 9.8 m/s\(^2\)

\( d \): diameter of the particle = \( 2 \times 10^{-6} \) m

The separated supernatant was washed at least two times with distilled water to remove the traces of dispersant. The previously described sedimentation process was applied at least three times successively on each clay fraction until the mass ratio of the dispersed particle collected in the solution was found negligible with respect to the total amount of pristine material (i.e. 5 g).

### 3.2.2 Method-II: Classical NaCl Treatment

This classical treatment is well described in the Handbook of Clay Science [3]. In this method 30 g of raw bentonite was dispersed in 500 ml of 1M NaCl solution, stirred for overnight and centrifuged. The above procedure was repeated three times.

Finally the slurry was washed with de-ionized water until chloride free slurry was obtained as tested by AgNO\(_3\) solution. The treated sample was dried at 60 °C and the clay particle less than 2\(\mu\)m was separated by sedimentation technique according to the Stokes law of sedimentation. The purified clay was obtained by dispersing 5 g of dried treated bentonite in 1L de-ionized water and collecting the supernatant dispersion of particles less than two micron (<2\(\mu\)m) after the pre-calculated time (10
hr) and height (15 cm) at 25 °C and dried at 60 °C. The dried sample was grounded to obtain fine powder for experimental use.

3.2.3 Method-III: Soda Activation Followed by Sedimentation

To obtain pure sodium montmorillonite, the soda activation procedure was applied as described by Yildiz and Calimli [142] and further to separate the main clay particles from associated minerals, especially quartz, the simple sedimentation technique was used. The complete procedure is described below.

The bentonite sample was activated by sodium carbonate (Na₂CO₃). To determine the optimum activation dose, the soda with various mass ratios of 2, 3, 5 and 8 g per 100 g of each clay sample, labeled as K2, K3, K5, K8 for Karak, N2, N3, N4, N5, N8 for Nowshera and P2, P3, P5, P8 for Peshawar bentonite were added to 1000 mL boiling water. The dispersion was stirred slowly but continuously with Magnetic Stirrer at boiling temperature for 1h. After this activation, the dispersion was allowed to cool down to room temperature. This was further diluted with distilled water to get the dispersion with 5 g activated clay per 1000 mL of distilled water. This dilution is necessary to avoid gel formation. The diluted suspension was dispersed by stirring and plunging to maximum possible homogeneous suspension. It was allowed to stand for 24 hr to settle down the impurities especially quartz. After 24 h, the supernatant was decanted and the sedimentation process was repeated on the sedimented sample until the supernatant became clear i.e. all clay particles are possibly separated. The collected supernatants were centrifuged with 10000 RPM to separate the clay sample and dried in an oven at 60 °C. The dried sample was further ground to obtain fine powder for experimental use.
The raw and all the purified activated samples were characterized by X-ray Diffraction and CEC measurement to determine the activation dose at which the possible transformation of calcium bentonite to sodium bentonite occurred. The raw and the sodium bentonite powder obtained at specific ratio were further characterized by X-ray Fluorescence, Inductively Coupled Plasma-Atomic Emission Spectrometry, \( \text{N}_2 \) adsorption-desorption isotherm at \( T=77.35\text{K} \), Microbial test, Pharmacopeial tests (i.e. swelling volume, pH and Gel Formation).

### 3.3 Characterization Techniques

All the raw and purified samples were characterized by X-ray diffraction, Scanning Electron Microscope (SEM), X-ray fluorescence (XRF), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), \( \text{N}_2 \) adsorption-desorption isotherm at \( T= 77.35\text{K} \), Microbial test, CEC measurements, and Pharmacopeial tests (i.e. pH, Swelling capacity and Gel formation). The raw and purified bentonites were also studied by laser granulometry to determine the efficiency in separating the small particles from the whole materials.

#### 3.3.1 X-ray Diffraction Analysis

X-Ray diffraction (XRD) is one of the most important techniques, which has been used for many years to determine the mineralogy of clay mineral on the basis of its basal spacing [143-148]. X-rays are electromagnetic radiation. Its wavelength is approximately 1Å, which is about of the same order as the spacing between the atomic planes of crystalline solid. Each crystalline material has its own characteristic X-ray patterns, which is used as a fingerprint for its identification. In general, the whole X-ray diffraction patterns consist of series of reflections of different intensities.
with 20 values. Each reflection of the pattern must be due to some constituent of the sample. A reference powder diffraction data for example, the mineral Powder Diffraction Files (PDF) compiled by the Joined Committee on Powder Diffraction Standard (JCPDS) and published by the International Center for Diffraction Data is required for unknown minerals identification. For this purpose first of all the diffraction pattern is necessary to obtain by XRD. The test pattern is then compared directly for known materials. XRD technique is used by material scientists and mineralogists to identify, quantify and to study the structure of clay and clay minerals. The study of clay mineral by XRD was described in number of textbooks [7, 149-150].

Identification of different clay mineral especially expandable clay minerals in clay sample on the basis of simple comparison of diffractogram with reference data are uncertain as these mineral have variable d$_{00L}$ spacing that relate and depends upon the exchangeable cations saturation and relative humidity. The nonexpansible clay minerals are not sensitive to these factors. For that reason non-expansible clay mineral can be easily distinguished from expansible clay mineral by their unchanged d$_{00L}$ spacing in XRD patterns that have undergone different chemical treatment. The application of diagnostic criteria based on response to cation saturation, glycerol salvation and heat treatments are needed to distinguish among expandable clay minerals. Different species of expansible clay mineral respond differently to these diagnostic conditions. Based on these general characteristics of clay mineral, a system of distinguishing smectite from other clay minerals and also the quantitative mineralogical analysis by XRD are explained in this section.
i. Qualitative XRD Analysis

Clay mineral group’s identification by XRD is commonly based on basal spacing (d001) of samples in three different states: air-dry, saturated with ethylene glycol (EG), and heated at 500°C [151]. The expandable clay minerals, smectite, change their basal spacing with EG solvation. When air dried, these minerals will have a basal spacing d001, of 12 Å to 15 Å. After treatment with ethylene glycol or glycerol, the smectite expand to 17 Å or 18 Å. When heated, d001 drops to about 10 Å as a result of the removal of interlayer water.

Lithium saturation procedure can be used as a test to distinguish montmorillonite from other smectite. The method was first introduced by Hofmann-Klemen [152], but this criteria was improved by Lim and Jackson based on classical specimen and their thermal reaction with lithium [153]. Heating Li-saturated montmorillonite to 250°C overnight (Li-250°C clay) allowed them to be differentiated from all other smectite by their resistance to solvation with glycerol vapor for 16 hr at 90°C. If the smectite present in Li-250°C clay is montmorillonite, this peak will stay collapse after treatment with glycerol. If the smectite present in sample is other than montmorillonite, the basal spacing d001 of smectite peak will still expand after treatment with glycerol. Identification can be easy if the clay fraction i.e. ≤ 2 µm is first separated from the raw sample by minimizing contamination of non-clay minerals.

There are numerous techniques for preparing oriented samples for XRD examination [7]. Sedimentation onto a glass slide is commonly used for identifying clay minerals but this technique is inappropriate for quantitative analysis because of clay segregation during sedimentation on the slide.
The samples preparation procedures for qualitative and quantitative analyses are described as follow.

**Sample Preparation for Differentiating Clay Mineral Group (Smectite):**

For qualitative XRD analyses, the oriented samples of raw bentonite were prepared by deposition of their dispersion on glass slides. 5g of the clay sample was soaked in distilled water for 24 hours and dispersed using a magnetic stirrer for 30 min. The clay suspension was then diluted in a 500 mL cylinder, dispersed by plunger for 3-4 minutes and was allowed to settle. Aliquots of clay suspension was taken from above 10 cm depth after 6 h at temperature 25°C and the suspension was then placed onto a glass slide mounted in the Petri dish and dried to produce a stable substrate. These samples were further treated according to the section 3.3.1 for analysis. For each bentonite of KPK three sedimented samples were prepared on glass slides and allowed to dry slowly to produce orientated deposits. One from each group was heat treated for 4h at 500ºC, one from each group was left in a desiccators with ethylene glycol at 40ºC for 24 h, and third from each group was kept in a desiccators as untreated sample. X-ray diffraction patterns were obtained for all nine samples.

**Sample Preparation for Differentiating Montmorillonite from Other Smectite:**

For identifying the smectite clay mineral specifically montmorillonite used for pharmaceutical industry lithium saturation method was applied.

The Lithium saturated samples were prepared by transferring 50 mg of each bentonite sample (≤ 2µm size) separated as described in first para above into a 15-ml test tube and washed 3 times with aqueous 3 M LiCl and 2 times with 0.01 M LiCl in 90% methanol using centrifugation process. Slurry was prepared with water and spread over a glass slide. The clay was allowed to dry slowly at 25°C and then heated
overnight at 250°C in a muffle furnace. Such samples were hereinafter designated as "Li-250°C " clay. Curling of the clay film during heating can be prevented by wrapping thin bands of aluminum foil tightly around the edges of the clay-coated slide prior to heating [154]. However in my sample this was not needed. The Li-250°C clay slides were cooled in a desiccators. These were then placed in a sealed glass container containing glycerol for salvation in an oven at 90°C for 16 h. After this treatment the sample is designated as “Li-250°C-glycerol solvated”.

For qualitative analysis, the XRD patterns of all oriented samples were recorded by Siemens D500 diffractometer equipped with a graphite monochromatic, and Cu Kα radiation at 40 kV and40 mA. The samples were scanned for 20 from 2–70° with a step scans of 1s per step of 0.02°.

ii. **Quantitative Analysis of Bentonite by Rietveld Method**

The quantitative analyses of the indigenous sample were performed by using Rietveld Method explained below.

**The Rietveld Method**

The Rietveld method is a whole pattern structural refinement approach to quantitative phase analysis using X-Ray powder diffraction data [155-157]. The main idea is that, on the basis of high quality powder diffraction data a diffraction pattern (called calculated diffraction pattern) is generated which is then can be simulated using Rietveld refinement software, and compared to the experimental diffraction pattern (called observed diffraction pattern). The software is then used to manipulate the simulated diffraction pattern by varying a number of parameters like site occupancies, atomic displacement parameters, unit cell co-ordinates, instrumental and experimental parameters and many others in order to match the experimental
diffractogram as closely as possible often using a least square minimization procedure. In Rietveld analysis, the quantity minimized in the least square refinement is the residual, $S_y$:

$$S_y = \sum_i w_i (y_{io} - y_{ic})^2$$

(3.1)

Where $w_i$ is the weight at point $i$ in the diffraction profile, $y_{io}$ and $y_{ic}$ are respectively the intensities observed and calculated in the $i$th step of the diffractogram and the sum is over all data point.

The value of calculated intensity at each point is given by:

$$y_{ci} = s \sum_k L_k |F_k|^2 \phi(2\theta_i - 2\theta_k) P_k A + y_{ib}$$

(3.2)

Where

$s$= Scale factor

$K$= Miller indices $h$, $k$ and $l$ for particular Braggs reflection

$L_k = (L_p M)$- where $L$, $P$, $M$ respectively represent Lorenz, polarization and multiplicity factors.

$\Phi$= Reflection profile function

$P_k$ = Preferred orientation function

$A$= Effective absorption factor

$F_k$= Structure factor for $K^{th}$ Braggs reflection

$y_{ib}$= The background intensity at the $i$th step.

It is important to keep in mind that to refine a crystal structure to obtain the adjustment profile is not only limited to vary the parameter randomly, but requires knowledge of the sample and strategy of refinement. The analysis of the values
obtained for the parameters is also necessary to evaluate whether they have physical sense [158].

During the refinement there are some quality factor called R- values that can be tracked in order to investigate that the refinement is converging to a minimum or diverging [158]. The ‘weighted pattern’ $R_{wp}$ is probably the most important and meaningful factor, since of purely mathematical point of view as defined by equation 3, it is the numerator’s residual being minimized.

$$R_{wp} = \sqrt{\frac{\sum w_i (y_{io} - y_{ic})^2}{\sum w_i (y_{io})^2}}$$ (3.3)

The meanings of the symbols are already explained in the previous paragraphs. The Rietveld method decreases the sum of the square of the difference between the calculated and the observed diffraction pattern. During refinement, if the value of $R_{wp}$ decreasing it means that the refinement is converging and the function is reaching a minimum value. However, if increasing means the values are diverging. If diverging, it is better to change the structure refinement strategy[158]. The choice of parameters which are to be fixed or refined (varied) have to be carefully considered as the simultaneous refinements of some parameters can easily lead to very bad fitting statistics or ill-considered conclusion.

The others important R- values are.

R expected factor,

$$R_{Exp} = \frac{(N - P)}{\sqrt{\sum w_i (y_{io})^2}}$$ (3.4)
Where \( N \) is the number of observation i.e. number of \( y_i \)'s are used, and \( P \) is the number of parameters being refined. The 'Pattern factor', \( R_p \) is given by

\[
R_p = \frac{\sum |y_{io} - y_{ic}|}{\sum y_{io}} \quad \text{(3.5)}
\]

The goodness of fit (GOF) is another useful indicator as defined by equation 6.

\[
\text{GOF} = \frac{R_{wp}}{R_{exp}} = \sqrt{\frac{\sum w_i (y_{io} - y_{ic})^2}{(N - P)}} \quad \text{(3.6)}
\]

In general, the GOF value of 1.3 or less is usually considered being quite satisfactory. However, the value close to 1.3 means that the \( R_{wp} \) reached the statistically expected value (\( R_{exp} \)) for that data.

Although numerical criteria are very supportive, it is important to use graphical tools including things like the difference plot, the calculated and observed profile, peak shape, etc. These plots and indicators give one a good path to follow in finding the cause of initial problems which one might be having in the early stages of a refinement. Gross errors in the scale factor, lattice parameter, zero offset, incorrect structure, phase contamination, etc are usually uncovered immediately in the plots, but not in the tables of numbers that are output from the refinement. The quantitative analysis of phases is determined according to equation 7.

\[
W_p = \frac{S_p(ZMV)_p}{\sum_{i=1}^{N} S(ZMV)} \quad \text{(3.7)}
\]
In equation (7) p refer to the phase whose amount is determined in the mixture of N phases, I refers to each of N phases, S is the scale factors derived from Rietveld refinement, Z is the formula units per unit cell, M is the mass of the unit cell in atomic mass units and V represent the volume of the unit cell [156,159].

Sample preparation for quantitative analysis:

For mineral phase quantification, the sample was prepared by random powder method and XRD pattern was recorded in the same condition except for angular range 2–90° 2θ and counting time 10s per step of 0.02°. The Rietveld method was used to quantify the clay sample by using Topas academic software [160].

3.3.2 X-Ray Fluorescence (XRF)

XRF is a useful analytical technique used to identify and determine the concentrations of elements present in the sample. XRF works, on the basis of detecting of the emitted x-ray from the excited atoms. This technique is a three step process begins with the excitation of the atoms by absorbing the high energy x-rays coming from external source. The second step is the transitions of electron from higher energy states to lower energy state and radiate secondary x-rays. The x-rays produced by this transition process are known as X-ray fluorescence [161]. This fluorescence x-rays are the characteristic x-ray of the element. The third step is the detection of fluorescence photon. The detector recorded the spectrum of these x-rays containing peak. The qualitative and quantitative information of the elemental composition can be obtained by measuring the energy of the x-ray peaks in the spectrum and the intensity of the various elemental peaks. The elemental
compositional analysis of bentonite is essential and useful for scientific and industrial application.

The major elemental compositions of Pakistani bentonite were determined through X-ray fluorescence (XRF) for the most abundant elements, with their relative oxides. The sample preparation included grinding and sieving, followed by the fusion with Lithium Borate. The analysis was performed through Philips X-ray fluorescence spectrometer, model PW2400. The spectrometer was calibrated using international standard reference materials suitable for sediments and rock material.

3.3.3 **Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)**

ICP-AES is another most important technique currently used for analysis of major and trace elements of clay sample because of its high accuracy multi element capability, good detection limit and simpler operation. In this technique the plasma source is used to dissociate the sample into its constituent atoms or ions, and exciting them to a higher energy level. They return to their ground state by emitting photons. This photon energy is the characteristic of the element present. These photons are recorded by an optical spectrometer. When calibrated against standard the technique provides a quantitative analysis of the running sample.

In this study, the trace elements (Pb, As & Cd) were measured in triplicate by using inductively coupled plasma-atomic emission spectrometry (ICP-AES), Spectro Brand Model Arcos, SOP. 100mg of samples were weighed in platinum crucibles previously decontaminated with 40ml of concentrated nitric acid, and then added acid with the following two different ratio named Test-1 and 2.

Test1: 3 ml of concentrated nitric acid and 2 ml of deionized water
Test2: 5ml of hydrofluoric acid, 3ml of HCl and 1 mL nitric acid and 2 ml of deionized water

The samples were then heated at 100 °C for 2 hours on a heating plate and then they were transferred to 25 ml flasks. The presence of different trace elements were then identified by ICP-AES, by determining the wavelength of the emitted radiation (As = 214.438 nm, Cd = 189.042 nm and Pb = 220.353 nm) and the concentration was calculated by measuring the intensity of the radiation.

3.3.4 Thermogravemetry and Derivative Thermogravemetry

The Thermogravimetric analysis (TGA) or thermogravimetry (TG) is an experimental technique widely used in clay analysis. This technique has been defined by ICTAC (the International confederation for thermal analysis and Calorimetry), as a technique in which the weight change of a substance is measured against temperature or time while the substance is subjected to a controlled temperature program [162]. The weight loss in TG curve only appears where the loss of unstable component occurs. The TGA results are presented as a plot of mass (m) against temperature (T) or time (t). In this plot the mass loss appear as a step. The steepest part of this plot presents the higher mass loss and vice versa.

The TG results can also be presented by alternate method called Derivative Thermogravemetry (DTG). It is a technique yielding the first derivative of the TG curve with respect to either time (dm/dt) or temperature (dm/dT), and to plot that against temperature or time [163]. The DTG curve plays an important role in differentiating the overlapping reaction to give double peaks or a shoulder on a main peak. A gradient change in the DTG curves appears due to superimposition of slow reaction with other fast reactions. The positions of the DTG peaks have no other
significant but to show where the mass loss is fastest. However in clay analysis these
peaks can be used as a finger prints to differentiate the minerals found in it.

In this study the aim of this experiment was to investigate the thermal properties
of the bentonite samples modified and purified by different methods and to determine
the differences of the thermogravimetric curves obtained for the bentonite purified
with different methods and raw bentonite sample.

Thermogravemetric analysis (TGA/DTG) of raw and purified bentonite samples
were performed by Shimadzu model TGA-51, in the temperature range of the
environment to 900°C. All experiments were carried out by placing about 20 mg of
samples under the dynamic atmosphere of air (50ml min-1) with a constant heating
rate of 10°C min⁻¹. The program TA 60 was used to determine the temperatures
involved in thermal events.

3.3.5 Cation Exchange Capacity (CEC) Measurements

In order to maintain the electro neutrality of clay particle charges arises from the
isomorphic substitution of Al⁺³ by Mg⁺², Fe⁺² in octahedral and/or Si⁺⁴ by Al⁺³ in
tetrahedral layer, cations such as Mg, Ca, Na, K are held in between the sheets, on the
surface and on the edges of clay particle. “The exchangeable and readily available
cations to be replaced by similar or other type of cations under different
environmental condition are quantified in terms of the CEC of clay” [49]. The
maximum quantity of exchangeable cations is termed as Cation Exchange Capacity of
the clay mineral, expressed as miliequlelents (meq) per 100 grams of dry clay mineral.
The CEC of montmorillonite clay mineral is the second highest (60-130meq/100g)
among all clay minerals, due to their high isomorphic substitution within the
octahedral layer, which results a net negative charge. In case of smectite mineral the interlayer cation is about 80% and the cation at crystal edge at pH-7 contributes 20% of the total cation exchange capacity [50]. There are many methods to calculate CEC but we are using the Ammonium Acetate Method at pH-7 which is still very familiar and also are used to verify the new development methods for CEC.

i. Determination of Cation Exchange Capacity by Ammonium Acetate Method at pH-7

The technique utilized for obtaining the cation exchangeable capacity (CEC) values of samples was based on ammonium acetate method [164]. In this method the CEC calculation is summarized in three steps: Sample preparation, distillation and titration.

ii. Sample Preparation

5.0 g of sample was dispersed in 200 mL of 3M ammonium acetate solution of pH 7.2 and stirred magnetically for 12 hours at room temperature. The dispersion was then allowed to stand for overnight. Next day the supernatant was carefully decanted, and the sedimented clay along with 30mL of absolute ethanol was transferred to 50mL centrifuge tube and centrifuged for 15 minutes with 3500 RPM. After centrifugation the supernatant was decanted, the centrifugation process was repeated thrice. The sedimented clay was dried at 60 °C, overnight. After drying Kjeldahl distillation process was used to determine the CEC. In this study The Kjeldahl Nitrogen Analyzer, model MA 036/Plus from Marconi Company was used.

The 1.5 g of treated clay as described above along with 50 mL of distilled water and 1ml of phenolphthalein were transferred to a Kjeldahl tube. The Kjeldahl tube was then attached with analyzer. The tube was attached to analyzer and added 50%
NaOH solution drop wise to the clay dispersion until the appearance of pink color. The distillation step was started and the distilled was collected in a recipient with 50 mL of boric acid mixed buffer, which was titrated with 0.1N hydrochloric acid solution. The volume of HCl used in titration was taken to determine the CEC of the samples by applying the following relation.

$$\text{CEC} = \left[ \frac{N \times V_{\text{HCl}} \times 100}{m} \right] \text{meq/100}$$

Where

- $N = \text{Normality of standard acid (HCl)}$
- $V_{\text{HCl}} = \text{volume of acid (HCl) used for titration}$
- $m = \text{mass of the sample in gram}$

The CEC of each sample was calculated in duplicate and the average of the two values was taken as a CEC of the sample.

3.3.6 Scanning Electron Microscopy

For the last 15 years, there has been a great development and improvement in the methods and optical equipments, used in the characterization of solid materials, especially clay minerals in clays and soils. The electron microscopy is the best technique for the examination of surfaces and textures analysis [165].

In order to study the morphology of my case study of indigenous bentonite samples, Scanning electron microscope was used. The basic principle of the technique is documented in the literature [166]. An electron gun shoots a narrow electron beam against the sample’s surface while it is under vacuum. Three magnetic lenses compress the size of the beam so that the size of the area being scanned reduces. On
impact with the sample the electron are reflected in a way that depends on the
topography of the constituents of the sample and a receiver-decoder of the reflected
electron produces a signal, which is converted into magnified images of the area being
scanned. The LEO equipment, LEO 440i was used for the current analysis. The air-
dried powders were used for testing. The powder of each sample was fixed on the
alumina stub by an adhesive carbon tape and coated with a thin layer of gold (to be a
good conductor of electrons). Each sample was then fixed in the sample holder and
the micrograph needed to analyze the morphology of the sample was obtained through
a scanning electron microscope (SEM) LEO 440i.

3.3.7 Particle Size Analyzer

The particle size analyzer Mastersizer 2000 is the most widely used in particle
size analysis. The Mastersizer 2000 is capable of measuring the particle size of
sample between 20 nm and 2000 µm and it work is based on the principle of laser
diffraction. In order to study the particle size distribution with a most appropriate way
Mastersizer 2000 equipped with aqueous dispersion unit (Hydro 2000MU)
manufactured by Malvern instruments Ltd, UK was used. The dispersion unit
contained a beaker of 600 to 1000mL capacity with a variable speed stirrer (0 to
4000rpm) and an ultrasonic mixer. The aqueous dispersion unit is very suitable for
clay mineral to be highly dispersing the clay aggregates before analysis.

Each sample separately added in portion to the aqueous dispersing unit until the
obscurcation reached to 10% and then sonicated for a short time so that the particle of
clay highly dispersed before injection to the instrument for analysis. The suspension
of each sample was injected into the analyzer by auto-mechanism and the size
analysis was recorded three times for each sample and the average was taken as final
result. The data for particle size and the percentage distribution for relative size are plotted in Chapter fig 4.4 to fig 4.6.

3.3.8 Surface Area and Pore Size Distribution

The surface area and Pore size distribution were determined by Micromeritics ASAP 2010 apparatus, using nitrogen as adsorbate at the liquid nitrogen boiling point T=77.35K. The setup was controlled by already ASAP 2010 software installed computer. With this software the specific surface area ($S_{BET}$), Pore size distribution and micropore volume/area were estimated by the Brunauer, Emmett and Teller (BET) method [167] with the cross sectional area 0.1620 nm$^2$ of nitrogen was always taken [72], Barrett Joyner-Halenda (BJH) model [168] and t-plot method [169] respectively. The value of pore size distribution gained from Adsorption branch of the isotherm was taken into account since desorption branch of isotherm profile is not suitable due to the blocking of nitrogen in bottle shaped pores [72].

3.3.9 Microbiological Analysis

The microbial test was carried out, by using plate dilution method [28].

i. Total Aerobic Microbial Count

Ten gram of each bentonite sample was suspended in 100mL of phosphate buffer and adjusted to pH-7.2. Serial dilution of $10^2$, $10^3$, $10^4$ etc were made of each sample in similar way. 1mL of each dilution of bentonite samples (in duplicate) was poured in sterilized Petri dishes. The soybean-casein digest agar medium (oxide) used as a culture media was sterilized and cooled back to 45 °C. 15 mL of the sterilized media was added to each plate. The plates were then shaken and left undisturbed for some time to solidify. After that the plates were inverted and incubated for 48 to 72 h, at 37
°C. The plates comprising 30 to 300 colonies were taken into consideration while the other plates were rejected. The average of the counted colonies for the two plates were calculated and multiplied by dilution factor and expressed as colony forming unit per gram (cfu/g) of sample

ii. **Test for E. Coli and Salmonella Species**

A volume of Fluid Lactose medium was added to the bentonite sample contained in a vessel to make 100 mL and incubated at 37 °C. The media was examined for growth and then mixed by shaking gently. 1 mL of this Pre-enriched culture media was transferred into two vessels having respectively 10 mL of Fluid Selenite-Cystine and Fluid Tetrathionate media, mixed, and incubated for 12 to 24 h at 37 °C.

For Salmonella species Streaked portions from both the Selenite- Cystine and Tetrathionate media were placed on the surface of a bismuth sulfite agar medium and a xylose-Lysine-Desoxycholate agar medium.

For E. coli, a portion from the remaining Fluid Lactose media was streaked on the surface of MacConkey agar medium containing in the Petri dish. All the Petri dishes were incubated at 37°C for 24 h.

The plates were examined after incubation for the presence of the characteristic colonies of Salmonella species and E.Coli.

**3.3.10 Pharmaceutical Tests**

In pharmaceutical uses, it is crucial importance that bentonite must fulfill some feature before considering their use in pharmaceutical sector, including high chemical, mineral and microbial purity. Specific characteristic termed as pharmacopeia
suspension test such as gel formation and swelling power are also important for some pharmaceutical application especially their use as suspending and disintgrant agent.

The pharmaceutical tests such as pH, sedimentation volume or gel formation and swelling property of the Raw and Purified bentonite were determined according to the methods described below.

i. **Swelling power**

The swelling power was determined according to the US Pharmacopeia method [25]. 2 g bentonite sample was added in portion to the 100 mL graduated cylinder containing 100 mL distilled water. The swelling capacities were then measured by quantifying the apparent sediment volumes after 2 h.

ii. **Gel Formation**

The technique based on European pharmacopeia was used for sedimentation volume or gel formation measurement [27]. The suspension of 6 g of each bentonite sample in 200 ml of water was prepared with a high-speed mixer at 10,000 RPM. 100 ml of each suspension was then added to 100 mL graduated cylinder and placed undisturbed. The sedimentation volume was then measured by quantifying the clear supernatant volume after 24 h.

iii. **pH measurement**

The pH value was measured with portable pH meter. The instrument was standardized with buffer solution of known pH (4 and 9). The electrode (probe) was washed with distilled water and drops were removed from the tip of the electrode with tissue paper. The probe was inserted into the clay water suspensions (4 g/200 ml) and the display reading of the pH meter was recorded [25].
Chapter-4

RESULTS AND DISCUSSION

This chapter presents the results of the case study of samples collected from three different mines located at different site of KPK province of Pakistan were characterized before and after purification. The observation for phase identification, mineral quantification and purification affect was estimated from XRD patterns. XRF data for Major elemental analysis and ICP-AES data for trace elemental analysis was used. Morphological analysis and particle size analysis were evaluated from SEM micrograph and Mastersizer data. N2- adsorption and desorption for surface and pore analysis and Ammonium acetate method at pH-7 using Kjeldahl distillation process for CEC measurement. The pharmacopoeias suspension properties (i.e. Sedimentation volume, swelling power and pH) were quantified from the results obtained by using US-Pharmacopeia quoted Method. Plate dilution method were used for microbiological analysis.

A group of six samples were prepared from each raw sample by soda activation with different mass ratio and purified by sedimentation method (Method-III). The completely transformed sodium bentonite was then confirmed from the X-ray diffraction patterns and CEC. As sodium bentonite is needed for further study therefore the rest of the activated samples were then discarded. Hereafter, we present the XRF, ICP-AES, SEM, Mastersizer, Micromeritics ASAP 2010 and other pharmacopeia suspension tests data on the three totally converted sodium bentonite samples.
Similarly three samples which were purified by classical method (Method-II) and three sample prepared by simple sedimentation were also studied for other properties through XRF, ICP-AES, SEM, Mastersizer, Micromeritics ASAP 2010 and other pharmacopeia suspension tests.

The data for all the samples purified by the three different methods (Method-I, II, III) were presented for comparison with one another and with the data for their corresponding raw sample.

### 4.1 Mineralogical Composition and Affects of Purification and Soda Activation

This section present the qualitative XRD spectra analysis, quantitative phase analysis, XRD analysis of samples purified by method-I and Method II, and XRD analysis of soda activated samples followed by sedimentation method (Method-III).

#### 4.1.1 Qualitative XRD Spectra Analysis

The diffraction pattern of the untreated; ethylene glycolated, heated and Li saturated samples are shown in the Figure 4.1-4.3. The x-axis shows the 2θ in degree and the y-axis presents the detected x-ray intensity. The vertical linear scale here only refers to the untreated clay sample; the subsequent patterns for other vertically shifted for comparison.

Following the procedure explained in section 3.3.1 (i), the expansion and collapsing of the basal spacing d_{001} for the Karak clay from d=15Å to d=17.1 Å, 9.7 Å for glycolated sample and for sample heated at 500°C for 4 hours respectively was observed (fig.4.1). For Nowshera clay (fig 4.2) the d=15.2 Å for air dried, expanded to d= 17.2 Å, by ethylene glycolate and collapsed to d=10 Å, by heating effect. The d
spacing of Peshawar clay (fig 4.3) changed from 14.6 Å to 16.5 Å by ethylene glycol and 10 Å by heating effect. Moreover no change was observed in the basal spacing 
\(d_{001}=10\ \text{Å}\) of air dried sample of all clay, throughout the glycolated and heating effect while basal spacing 
\(d_{001}=7.2\ \text{Å}\) of air dried clay samples was disappeared after heating clay samples at 500°C for 4 h showed the presence of illite and kaolinite clay minerals respectively. Thus, the results confirm that the material \(\leq 2\ \text{µm}\) of the bentonites selected are composed mainly of smectite minerals and small amount of illite. In addition to this, kaolinite was also detected in Karak bentonite.

Fig. 4.1: XRD pattern of <2 µm fraction of Karak bentonite (a) Untreated (b) Ethylene glycolated (c) Heated at 500°C (d) Li-250°C-glycerol solvated. The basal spacing \(d\) (00l) of montmorillonite, kaolinite and illite are labeled as 001M, 001K, 001I.
Fig. 4.2: XRD patterns of <2 µm fraction of Nowshera bentonite (a) Untreated (b) Ethylene glycolated (c) Heated at 500°C (d) Li-250°C-glycerol solvated. The basal spacing d(001) of montmorillonite and illite are labeled as 001_M, 001_I.
Fig. 4.3: XRD pattern of <2 µm fraction of Peshawar bentonite (a) Untreated (b) Ethylene glycolated (c) Heated at 500°C (d) Li-250°C-glycerol solvated. The basal spacing d(001) of montmorillonite and illite are labeled as 001_M and 001_I respectively.

The XRD results of “Li-250°C-glycerol solvated” sample of Karak, Peshawar and Nowshera bentonites show the same irreversible collapsing effect to 9.6 Å after glycerol solvation at 90°C for 16 h (fig 4.1-4.3). This collapsing of the d001 basal spacing of all the studying samples reflects that this peak corresponds to montmorillonite of smectite clay group minerals [153].
4.1.2 Quantitative Analysis Using Rietveld Refinement

Rietveld method was applied to refined XRD pattern obtained from the indigenous bentonite samples for mineral phase quantification with the Rietveld Program TOPAS academic. Fig. 4.4 to fig. 4.6 shows the illustrations of the experimentally collected and the Rietveld calculated profile of the three raw samples after structures refinement. In these figures the red line represent the raw experimental XRD diffraction pattern, the blue line stand for calculated (refine) pattern from Rietveld analysis, and below these patterns the difference plot is shown in grey. The quality factor called different R-value are calculate from the calculated and observed x-ray pattern through software (TOPAS academic) and presented in Table 4.1. The Quality factor and Goodness of Fit (GOF) are explained in section 3.3.1 (ii). As seen from the fig 4.4 to fig. 4.6 that the calculated diffraction pattern are closely fit to the observed diffraction pattern and also R values are quite satisfactory as the GOF value are very close to 1.3 which is one of the accepted and reliable condition.

<table>
<thead>
<tr>
<th>Sample</th>
<th>R_p</th>
<th>R_wp</th>
<th>R_exp</th>
<th>GOF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karak</td>
<td>8.096</td>
<td>11.408</td>
<td>6.605</td>
<td>1.727</td>
</tr>
<tr>
<td>Nowshera</td>
<td>8.231</td>
<td>11.721</td>
<td>6.865</td>
<td>1.707</td>
</tr>
<tr>
<td>Peshawar</td>
<td>7.530</td>
<td>10.209</td>
<td>7.445</td>
<td>1.371</td>
</tr>
</tbody>
</table>

The scale factor, which is a variable to match as closely as possible the calculated and observed intensity of diffractogram, derived from Rietveld method is then used to calculate the presence of different mineral quantitatively as given by equation 3.7. The results of quantitative phase analysis are given in the Table 4.2.
Table 4. 2: The quantitative phase analysis in weight % of indigenous raw samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Montmorillonite</th>
<th>Kaolinite</th>
<th>Illite</th>
<th>Albite</th>
<th>Quartz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karak</td>
<td>74.37</td>
<td>8.83</td>
<td>12.37</td>
<td>-----</td>
<td>3.93</td>
</tr>
<tr>
<td>Nowshera</td>
<td>67.67</td>
<td>-----</td>
<td>0.32</td>
<td>17.70</td>
<td>14.32</td>
</tr>
<tr>
<td>Peshawar</td>
<td>77.37</td>
<td>-----</td>
<td>0.18</td>
<td>16.38</td>
<td>6.07</td>
</tr>
</tbody>
</table>

These results indicate that all Pakistani bentonite are rich in montmorillonite but having different concentration of other clay minerals (i.e. kaolinite, illite) and non clay mineral (albite, quartz), which obviously will present different behavior while using them in different industrial sector. It is important to mention that the Karak
bentonite is comparatively rich in clay mineral as it have negligible amount of non-clay mineral i.e. 3.93% quartz.

According to viseras et al. [22], the quartz above 2% in bentonite should be controlled as it offers limited evidence of carcinogenicity in human. Therefore the presence of high content of quartz i.e. greater than 2%, in Pakistani bentonite should be reduced to possible extent before their use in pharmaceutical sector.

Fig. 4.5: Rietveld refinement graph of Nowshera raw Sample.
4.1.3 XRD Analysis of Sample Purified by Method-I and Method-II

Due to the presence of high content of crystalline silica i.e. quartz, the purification step was believed unavoidable before its safe use in pharmaceutical sector. The sample were purified by different method i.e. Simple sedimentation using sodium hexametaphosphate as dispersing agent in Method-I and sodium chloride treatment followed by sedimentation to get pure sodium bentonite in Method-II. Here we want to investigate the difference between the two methods and the effect of NaCl treatment and the purity of the purified samples. It is well known that montmorillonite
with sodium interlayer cations have the basal spacing (d001) of about 12.5 Å in its air
dried state [5-6].

The XRD patterns of raw and the samples purified by method-I &II of Karak
bentonite are presented in fig. 4.7. The raw sample contains quartz clearly indicated
by the vertical line as shown in fig 4.7a. The other major peak contain in raw samples
are montmorillonite, kaolinite and illite represented by d001_M, d001_I and d001_K
having d-spacing 15 Å, 10.5 Å and 7.2 Å respectively. No variation was observed in the
d-spacing of clay mineral peak but the quartz peak was completely disappear in
sample purified by method-I as shown in fig 4.7b. The results observed for sample
purified by method-II was not the same as the sample purified by method-I. In case of
purification Method-II, not only the quartz peak was found disappear but the d-
spacing of montmorillonite d001_M was also reduced to 12.5 Å (fig.4.7c). This reduction
of d-spacing effect reflects that the Na^+ ions replaced Ca^{2+} ions in the montmorillonite
interlayer and have been changed into sodium forms.

Similarly the quartz peak was also observed in raw samples of Nowshera and
Peshawar bentonite (fig 4.8a and fig 4.9a). The clay mineral peaks in both Nowshera
and Peshawar bentonite were denoted by d001_M for montmorillonite d001_I for illite.
The non clay mineral albite was also observed in Nowshera and Peshawar raw
samples. In both the purification methods (Method-I & II), the peak of albite and
quartz was found absent and the basal spacing of montmorillonite of samples purified
by method II was decreased. It was 12.6 Å in Nowshera (fig.4.8c) and 12.8 Å in
Peshawar bentonite (Fig.4.9c). This decrease in the d-spacing of major peak of
montmorillonite is the confirmation of conversion of Ca+-montmorillonite to Na+-
montmorillonite.
Fig. 4.7: XRD diffractogram of Karak bentonite (a) Before purification (b) Purified by Method-I (c) Purified by Method-II. The basal spacing (d00l) of montmorillonite, kaolinite and illite are labeled 001_M, 001_K, 001_I.
Fig. 4. 8: XRD diffractogram of Nowshera bentonite (a) Before purification (b) Purified by Method-I (c) Purified by Method-II. The basal spacing d(00l) of montmorillonite is labeled 001_M.
Fig. 4.9: XRD diffractogram of Peshawar bentonite (a) Before purification (b) Purified by Method-I (c) Purified by Method-II. The basal spacing d(001) of montmorillonite is labeled 001\textsubscript{M}.

4.1.4 XRD Analysis of Soda Activated Sample Followed by Sedimentation (Method-III)

The raw clays were activated with soda (Na\textsubscript{2}CO\textsubscript{3}) with various mass ratios at boiling temperature for one hour and then sedimentation technique was used. These samples were dried and were grounded to soft powder. The XRD pattern for each sample activated by different mass ratio of soda is taken and these are given in fig
4.10 to fig 4.12. The objective here was to see the optimum dose of soda ratio at which the possible transformation of raw bentonite occur to sodium bentonite.

The X-ray diffraction patterns of Karak raw and purified Na$_2$CO$_3$ activated samples are illustrated in fig.4.10. Montmorillonite is the main mineral, however, minor amounts of quartz, illite and kaolinite were also identified by XRD. These inorganic non clay impurities were removed by sedimentation after soda activation. As seen from the activated sample patterns, the peaks of quartz mineral were disappeared; the main montmorillonite hkl diffraction reflections are present in all Samples. Lower d(001) values of Karak sample were found for the Na$^+$-bentonite obtained by soda activation at 5 g Na$_2$CO$_3$/100 g bentonite (about 12.6 Å) than in the original Ca$^{2+}$-bentonite (15 Å) shown in fig 4.10.

In case of Nowshera sample, the detected non clay minerals (quartz & albite) peaks in raw bentonite were found absent in all the activated purified samples (fig. 4.11). The basal spacing of montmorillonite phase of raw sample (d(001)=15 Å) was reduced to lower value of 12.5 Å at 5g Na$_2$CO$_3$/100g bentonite fig. 4.11. Similarly the Peshawar raw sample was liberated from the non clay mineral such as quartz and albite, and the Basel spacing d001$_M$ = 14.6 Å were decrease to smallest possible value of 12.7 Å at 3g soda/ 100g activation dose (fig.4.12).

The above discussion results that the Na$^+$ ions replaced the pre-existing cations upto possible extend in the montmorillonite interlayer in all the three raw samples and have been changed into sodium forms but at different activation dose. For Karak and Nowshera bentonite the optimum soda activation dose for transformation of raw bentonite to sodium bentonite was 5g soda/100g of dried clay while for Peshawar bentonite it was 3g soda/100g of dried clay.
Fig. 4.10: XRD diffractogram of Karak bentonite (a) Before activation (b) Activated with 2% Na₂CO₃ (c) Activated with 3% Na₂CO₃ (d) Activated with 5% Na₂CO₃ (e) activated with 8% Na₂CO₃.
Fig. 4.11: XRD diffractogram of Nowshera bentonite (a) (a) Before activation (b) Activated with 2% Na₂CO₃ (c) Activated with 3% Na₂CO₃ (d) Activated with 5% Na₂CO₃ (e) activated with 8% Na₂CO₃.
Fig. 4.12: XRD diffractogram of Peshawar bentonite (a) Before activation (b) Activated with 2% Na$_2$CO$_3$ (c) Activated with 3% Na$_2$CO$_3$ (d) Activated with 5% Na$_2$CO$_3$ (e) activated with 8% Na$_2$CO$_3$.

4.2 Cation Exchange Capacity

The CEC is one of the most important properties of bentonite for its use in pharmaceutical sector. We want to measure the CEC of all the raw, purified and
activated samples but first of all the CEC of all the soda activated bentonite sample were investigated in order to find out the optimum dose of soda activation at which the conversion of raw bentonite into sodium bentonite occur. Although XRD is the standard technique for complete transformation but to have double check we also measure the cation exchange capacity to conform the montmorillonite transformation by soda activation. For this confirmation purpose and also to know the CEC value of raw and activated samples we measure the CEC value of all the soda activated samples prepared with different soda mass ratio in chapter-3 for each raw bentonite. The CEC was determine for raw and all purified and activated samples were measure through Ammonium Acetate Method using Kjeldhal distillation process as explained in chapter-3.

4.2.1 CEC of Raw and the Soda Activated Samples

The CEC of raw and the soda activated samples of Pakistani bentonite are explained as follow.

i. CEC of Karak Raw and Activated Bentonite

The CEC (meq/100g) of Karak bentonite samples before and after activation by Na$_2$CO$_3$ with different ratios are shown in Table-4.3. The CEC of raw bentonite was 80meq/100g. The increase in CEC value was observed with the increase of soda activation dose and reached to maximum value of 124 meq/100 at 5g Na$_2$CO$_3$ /100g bentonite. After this soda activation dose, there was no increase in CEC value detected. This means that Karak bentonite changed to sodium bentonite with the activation dose of
5% Na₂CO₃, consistent with the XRD results. The increase in CEC was achieved, not only due to high clay content obtained by purification but also by the soda activation effect which can be generally explained as follow.

In general, it is thought that ion exchange did not increase or decrease the CEC of bentonite but in soda activation process where the heat is involved as in our case the significant increase or decrease in CEC of some bentonite has been observed. For example Yiltz et al. [93] observed that CEC was decreased correspondingly by increasing soda ratio while Rasin &Hamad et al. [170] observed the increase in CEC with increasing soda ratio. In our case the CEC was increased as well. The XRD results confirmed the replacement of predominantly interlayer cations i.e. Ca²⁺, Mg²⁺ etc by Na⁺ cations as the d(001)M decreased to 12.6 Å by 5% Na₂CO₃. The chemical analyses of K₅ sample (named sample purified by Method- III) shown in Table 4.3, the content of iron oxide decreased and magnesium oxide slightly increased after activation and purification which is the indication of increase in clay contents as well as the free interlayer Mg²⁺ cations at each stage of soda activation may not comes out but substitute either for Al³⁺ in the octahedral sheet or for Fe³⁺ and Si⁴⁺ in the tetrahedral sheet of bentonite particle, which resulted in ionic deficiency and hence increase in CEC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raw</th>
<th>K2</th>
<th>K3</th>
<th>K5</th>
<th>K8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEC(meq/100g)</td>
<td>80</td>
<td>102</td>
<td>115</td>
<td>124</td>
<td>121</td>
</tr>
</tbody>
</table>
ii. **CEC of Nowshera Raw and Activated Bentonite**

In similar process the CEC values obtained for Nowshera bentonite samples before and after activation by Na$_2$CO$_3$ with different ratio are shown in Table 4.4. The CEC of raw bentonite is 87 meq/100g. The slight increase in CEC was observed till 5% of sodium carbonate addition. After this ratio, the CEC gives almost the constant value i.e. 96 meq/100g. This indicates that Nowshera bentonite changed to Na$^+$-bentonite at the ratio 5g Na$_2$CO$_3$ /100g clay. The observed change in CEC may be due to removal of impurities by sedimentation. The montmorillonite content separation increased with increase in soda ratio due to colloidal dispersion resulting from replacement of pre-existing interlayer cations by sodium cations. The best possible colloidal dispersion produced due to complete conversion of Nowshera bentonite to Na$^+$-bentonite at 5g Na$_2$CO$_3$/100g of clay and hence maximum possible separation of montmorillonite particle occur and gives the maximum value of CEC of 96 meq/100g.

Table 4.4: Cation exchange capacity (CEC) of the soda activated Nowshera bentonite samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raw</th>
<th>N$_2$</th>
<th>N$_3$</th>
<th>N$_5$</th>
<th>N$_8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEC(meq/100g)</td>
<td>87</td>
<td>90</td>
<td>94</td>
<td>96</td>
<td>96</td>
</tr>
</tbody>
</table>

iii. **CEC of Peshawar Raw and Activated Bentonite**

The CEC of raw bentonite was found 65 meq/100g. But it was observed that CEC increased significantly with different mass ratio of soda and become maximum at 3 g Na$_2$CO$_3$ which was found 89 meq/100 as shown in Table 4.5. After this activation dose
the CEC was seen almost the same. This increase in CEC is partly contributes from soda activation process and partly from purification by sedimentation enhancing the montmorillonite clay mineral contents. Like Karak bentonite the increase in CEC contributing from the activation with different soda ratio may be detected due to isomorphic substitution of Silicon by aluminum in the tetrahedral sheet and aluminum by Magnesium in the octahedral sheet of bentonite particle. This means that this increase in net negative charge by isomorphic substitution occur till the activation of 3g Na₂CO₃/100g which was balanced by sodium ions (Na⁺). Thus the excess negative charge produced with activation which resultantly increases the CEC to 89 meq/100g bentonite in Peshawar activated sample.

Table 4.5: Cation exchange capacity (CEC) of the soda activated Peshawar bentonite samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raw</th>
<th>P2</th>
<th>P3</th>
<th>P5</th>
<th>P8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEC(meq/100g)</td>
<td>65</td>
<td>79</td>
<td>89</td>
<td>89</td>
<td>88</td>
</tr>
</tbody>
</table>

4.2.2 Comparative Study of CEC for Purification Method-I, II&III and Among Different Raw Bentonite of KPK

In order to identify Method of purification and the source of bentonite for high CEC we plot here the CEC data for comparison. The CEC of raw and purified samples of studied indigenous bentonites are shown in fig 4.13. It was observed that, the CEC value of Nowshera and Karak raw bentonite were comparatively high than Peshawar raw
bentonite. The CEC value was observed to increase with all purification methods, but relatively high increase in CEC values were resulted in samples purified by Method-II and III. The slight increase in CEC in sample purified by method-II may be due to obtaining relatively more pure clay. The significant increase in Karak and Peshawar bentonite by method-III is may be due to isomorphic substitutions occurring at octahedral and tetrahedral sheets already explained in section 4.2.1. It is also very interesting to note that, the CEC of Karak sample is highly affected by purification in comparison to other indigenous samples as it was increased to 11%, 22% and 55% by Method-I, II & III respectively. Moreover, the increase in CEC for Nowshera and Peshawar bentonite after purification Method-I, II & III were from 4% to10% and from 6% to 37% respectively. The small increase in case of Nowshera bentonite by all the purification methods indicate that the ion exchange did not affects the CEC value. The change in CEC is highly dependent on the amount of montmorillonite contents in clay and the occurrence of isomorphic substitution at octahedral and tetrahedral sites.

On the basis of CEC results all the raw and purified samples are suitable as they gives results within the range of 60 to 130 meq/100g bentonite which is the required value for bentonite to be used as pharmaceutical raw candidate but it must comply with the basic requirement i.e. mineral, chemical, microbial purity. It is not out of place to mention that sample purified by method II and III may be preferred in some special cases such as drug control release system because of its high CEC.
4.3 Chemical Analysis

In this section the chemical analysis of indigenous bentonite before and after purification are presented. The chemical analyses are divided in two subsection named Major elemental analysis and trace elemental analysis. The most abundant elements with their relative oxides called major elemental analyses were determined through XRF. The trace elemental analyses were determined using ICP-AES.

4.3.1 Major Elemental Analysis

The experimental results for major elemental analyses of the indigenous raw and purified samples from three different sites are presented separately below.
Major Elemental Analysis of Karak Raw and Purified Bentonite

The major elemental analyses of raw and the samples purified by the three methods (discussed in chapter-3) are presented in Table 4.6. It was observed from the result that SiO$_2$ decreased while Al$_2$O$_3$ increased slightly in purified samples. The slight decrease and increase in Silica and aluminum oxide respectively confirmed the removal of minor amount of impurities such as quartz and a proportional increase in the clay mineral contents. This finding was consistent with the XRD results given in sections 4.1.3 & 4.1.4. Also the Na$_2$O content increased and the CaO content decreased significantly in samples purified by methods II and III which was in accordance to the conversion of calcium bentonite to sodium bentonite. The chemical analyses also showed that the contents of MgO slightly increased in sample purified by method-I and III while decrease in sample purified by Method-II. The decrease of MgO in sample purified by method II is more probably due to removal of interlayer Mg$^{+2}$ cation after replacement with Na$^+$ cation while in Method-III by the free interlayer Mg$^{2+}$ cations may not comes out but substitute either for Al$^{+3}$ in the octahedral sheet or for Fe$^{+3}$ and Si$^{+4}$ in the tetrahedral sheet of bentonite particle. The low percentage of K$_2$O and CaO contents were still found in purified sample (Method-II &III). The contents of K$_2$O in the purified sample is more probably related to illite while CaO to negligible amount of calcite or feldspar or both of them. The values of Fe$_2$O$_3$, TiO$_2$ and MnO were relatively low in all purified samples but significantly decreased in sample purified by Method-III which reflected to the purity and whiteness of the sample.
The Loss of ignition (LOI) was also significantly decreased in purified samples (Method-II and III) as observed in Table 4.6. This difference in LOI is directly related to the hydration energy of the exchangeable cations. The $\text{P}_2\text{O}_5$ value was decreased in all Pakistani purified samples (Method-II, III) except in samples purified by Method-I where this content increased relatively corresponds to the traces of sodium hexametaphosphate.

<table>
<thead>
<tr>
<th>Chemical oxide</th>
<th>Raw</th>
<th>Purified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method-I</td>
</tr>
<tr>
<td>$\text{SiO}_2$</td>
<td>59.59</td>
<td>58.63</td>
</tr>
<tr>
<td>$\text{Al}_2\text{O}_3$</td>
<td>17.99</td>
<td>18.89</td>
</tr>
<tr>
<td>$\text{Fe}_2\text{O}_3$</td>
<td>2.90</td>
<td>2.74</td>
</tr>
<tr>
<td>$\text{MgO}$</td>
<td>6.71</td>
<td>6.85</td>
</tr>
<tr>
<td>$\text{CaO}$</td>
<td>1.59</td>
<td>1.25</td>
</tr>
<tr>
<td>$\text{Na}_2\text{O}$</td>
<td>0.24</td>
<td>0.47</td>
</tr>
<tr>
<td>$\text{MnO}$</td>
<td>0.082</td>
<td>0.084</td>
</tr>
<tr>
<td>$\text{K}_2\text{O}$</td>
<td>1.02</td>
<td>0.89</td>
</tr>
<tr>
<td>$\text{TiO}_2$</td>
<td>0.329</td>
<td>0.272</td>
</tr>
<tr>
<td>$\text{P}_2\text{O}_5$</td>
<td>0.064</td>
<td>0.140</td>
</tr>
<tr>
<td>LOI</td>
<td>9.65</td>
<td>9.78</td>
</tr>
<tr>
<td>Total</td>
<td>100.17</td>
<td>100.00</td>
</tr>
</tbody>
</table>
even after three times washing by distilled water. But it is not worthless to mention that, the Na (PO₃)₆ is not harmful to human health. It is often used in pharmaceutical industry.

ii. Major Elemental Analysis of Nowshera Raw and Purified Bentonite

The major elemental analyses of Nowshera bentonite samples before and after purification by the three different methods are reported in Table 4.7. Not like Karak bentonite, the change in silica and aluminum oxide was observed; the silica oxide was slightly increased or remains the same as in raw bentonite. It does not mean that impurities were not removed such as quartz but it is directly related to the increase in clay mineral fraction after purification. The decrease in CaO and increase in Na₂O in sample purified by method-II and III indicate the replacement of predominantly cations by Na⁺ cation in the interlayer of montmorillonite clay mineral particle and have been changed the original bentonite to sodium form. The small contents of K₂O in the purified sample is more probably related to illite while CaO to small amount of feldspar or calcite or both of them. Like Karak bentonite not only the value of Fe₂O₃, TiO₂ was decreased in all purified samples but also the Loss of ignition (LOI) decreased significantly in samples purified by Method-II and III (Table 4.7).
Table 4. 7: Major elemental analysis of Nowshera clay before and after purification.

<table>
<thead>
<tr>
<th>Chemical oxide</th>
<th>Raw</th>
<th>Purified Method-I</th>
<th>Purified Method-II</th>
<th>Purified Method-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO₂</td>
<td>60.32</td>
<td>59.54</td>
<td>61.38</td>
<td>60.33</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>18.10</td>
<td>18.87</td>
<td>19.44</td>
<td>18.89</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>2.86</td>
<td>2.31</td>
<td>2.61</td>
<td>2.06</td>
</tr>
<tr>
<td>MgO</td>
<td>4.70</td>
<td>5.46</td>
<td>4.99</td>
<td>5.25</td>
</tr>
<tr>
<td>CaO</td>
<td>2.49</td>
<td>2.14</td>
<td>0.17</td>
<td>1.41</td>
</tr>
<tr>
<td>Na₂O</td>
<td>0.76</td>
<td>0.80</td>
<td>3.48</td>
<td>3.37</td>
</tr>
<tr>
<td>MnO</td>
<td>0.066</td>
<td>0.061</td>
<td>0.059</td>
<td>0.048</td>
</tr>
<tr>
<td>K₂O</td>
<td>0.83</td>
<td>0.41</td>
<td>0.50</td>
<td>0.24</td>
</tr>
<tr>
<td>TiO₂</td>
<td>0.335</td>
<td>0.232</td>
<td>0.250</td>
<td>0.171</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.081</td>
<td>0.135</td>
<td>0.016</td>
<td>0.010</td>
</tr>
<tr>
<td>LOI</td>
<td>9.14</td>
<td>9.72</td>
<td>6.50</td>
<td>7.65</td>
</tr>
<tr>
<td>Total</td>
<td>99.68</td>
<td>99.68</td>
<td>99.40</td>
<td>99.89</td>
</tr>
</tbody>
</table>

iii. Major Elemental Analysis of Peshawar Raw and Purified Bentonite

The results for major elemental analyses for Peshawar bentonite are presented in table 4.8. These results are different from Karak and Nowshera samples. Here relatively greater value of SiO₂ and smaller value of Al₂O₃ was observed in raw Peshawar bentonite than the other local raw bentonite (Table 4.8). The decrease and increase of SiO₂ and Al₂O₃ respectively was observed after different purification Method. The same value of
Fe₂O₃, TiO₂ and MnO content were observed in peshawer bentonite samples before and after the purification. This is expected to be, because this bentonite was relatively white, a reflection of lower contents of Fe₂O₃, TiO₂ and MnO in this bentonite [96]. The values of K₂O and CaO were also decreased significantly. It is interesting to mention that Peshawar raw bentonite have relatively larger value of Na₂O than Karak and Nowshera Bentonite. This sodium content was significantly increased in purified sample (Method-II and III) while slightly decrease in sample purified by method-I. This increase and decrease of Na₂O and CaO respectively, in purified samples (Method-II & III), confirming the conversion of Ca²⁺-bentonite to Na⁺-bentonite. Like Karak bentonite the contents of MgO slightly increased in sample purified by method-I and III while decrease in sample purified by Method-II. In Peshawar bentonite, the loss of ignition was almost the same as raw bentonite after purification. The value of P₂O₅ was decreased not only in sample purified by Method-II & III but also in Method-I indicating no traces of Na(PO₃)₆ after washing.
Table 4. 8: Major elemental analysis of Peshawar raw and the samples purified by the three methods

<table>
<thead>
<tr>
<th>Chemical oxide</th>
<th>Raw</th>
<th>Method-I</th>
<th>Method-II</th>
<th>Method-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO₂</td>
<td>63.42</td>
<td>61.53</td>
<td>61.65</td>
<td>61.44</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>15.84</td>
<td>17.16</td>
<td>17.31</td>
<td>17.51</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>2.27</td>
<td>2.53</td>
<td>2.43</td>
<td>2.31</td>
</tr>
<tr>
<td>MgO</td>
<td>3.70</td>
<td>5.03</td>
<td>4.49</td>
<td>5.30</td>
</tr>
<tr>
<td>CaO</td>
<td>2.67</td>
<td>2.25</td>
<td>0.38</td>
<td>1.45</td>
</tr>
<tr>
<td>Na₂O</td>
<td>1.14</td>
<td>0.92</td>
<td>3.99</td>
<td>2.46</td>
</tr>
<tr>
<td>MnO</td>
<td>0.045</td>
<td>0.044</td>
<td>0.043</td>
<td>0.043</td>
</tr>
<tr>
<td>K₂O</td>
<td>1.98</td>
<td>0.88</td>
<td>0.70</td>
<td>0.39</td>
</tr>
<tr>
<td>TiO₂</td>
<td>0.326</td>
<td>0.331</td>
<td>0.325</td>
<td>0.318</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.122</td>
<td>0.107</td>
<td>0.034</td>
<td>0.022</td>
</tr>
<tr>
<td>LOI</td>
<td>8.29</td>
<td>9.13</td>
<td>8.26</td>
<td>8.48</td>
</tr>
<tr>
<td>Total</td>
<td>99.80</td>
<td>99.91</td>
<td>99.61</td>
<td>99.72</td>
</tr>
</tbody>
</table>

4.3.2 Trace Elemental Analysis

The trace elemental analysis is one of the important parts of this project. Due to side effects of the toxic trace elements on human health, the use of clay products in health are legally bound [138-139]. But Attention must be paid to the amount of Pb and As subsisting in the Bentonite, which should not exceed the pharmacopoeial limits for “Bentonite” and “Purified Bentonite” being 40 and 15ppm for Pb and 5 and 3 ppm for As.
respectively [25-26]. Therefore it is very important to be tested the bentonite before its use in pharmaceutical sector.

In this study the trace elemental compositions were obtained by using ICP-AES. The trace elements were found to be very low therefore to make sure of the results; extreme care was taken to dissolve the sample completely. Solutions were made in two different concentration of acid solvent as explained in chapter-3. The instrument (ICP-AES) was also calibrated afresh with standard samples of weak concentration of trace elements before determining the concentration in our samples for Pb, As and Cd toxic trace elements. It was found that in all samples, the concentrations of elements were below the detection limit of the test equipment and it was well below the permitted range of major pharmacopeias. The values obtained for Pb, As and Cd in ppm for all studied samples before and after purification along with the permitted limit for Pb and As in US-Pharmacopeia are reported here in Table 4.9. From these results it was concluded that all the studied Pakistani bentonites are chemically free from Pb and As in both raw and purified form to a level of ≤ 0.05ppm and ≤ 0.01ppm respectively. These are too less than pharmacopical limit i.e. Pb ≤ 40 ppm and As ≤ 5 ppm for raw bentonite while ≤ 15 and ≤ 3ppm for purified bentonite respectively [25-26].
Table 4.9: Trace elemental analysis of all purified and untreated bentonites

<table>
<thead>
<tr>
<th>Elements</th>
<th>Results-1 (ppm)</th>
<th>Results-2 (ppm)</th>
<th>US- Pharmacopeia limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>≤40</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>-----</td>
</tr>
<tr>
<td>As</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>≤5</td>
</tr>
</tbody>
</table>

4.4 Particle Size Analysis

Like other physicochemical properties such as CEC, swelling power, surface area of clay mineral the small particle size is the another important characteristics of clay that make them a suitable candidate to be used as raw material in pharmaceutical industry, cosmetic or skin protective purposes [18,29]. Although for clay particle size, there is no generally accepted upper limit [3] but some discipline have conventionally set a maximum size of clay particles such as sedimentology, geology and geo engineering it is commonly restricted to 4µm in equivalent spherical diameter [7,35]. While in pedology smaller than 2µm and in colloid science the value of 1µm is generally accepted [3]. Now to evaluate our indigenous bentonite clay sample it is important to be tested for particle size analysis before using them for specific pharmaceutical use. The raw and purified bentonites were studied by laser granulometry to find out the range of particle size in our raw samples and also to determine the efficiency of the three different purification methods in separating the small particles from the whole materials.
i. Group of Samples of Karak bentonite

The raw sample of Karak bentonite showed bimodal particle size distribution, one with higher volume percentage centered at 4µm attributed to the clay particle and the other centered at 12µm showed the traces of non clay mineral particles such as quartz (Fig. 4.14). After purification by method-I, the volume percentage of the fine particle size increased dramatically while the particle larger than 4µm became negligible. The size distribution plot of Karak clay purified by Method-II gave particle size distribution with two maxima (one centered at 0.5µm and other centered at 1.5µm) attributed to the purity of the clay (fig. 4.14). After Soda activation followed by Sedimentation (method-III), the situation was very different, showed multimodal particle size distribution with 3 maxima (fig. 4.14), one with higher volume percentage centered at 0.2µm and other with lower percentage at 0.5 µm and 1.5 µm. However, the volume percentage of particle greater than 2 µm is very small as compared to raw and the sample purified by Method-I and II.

The purification process strongly affects the size distribution plot, shifting it to fine particle size which is attributed to the montmorillonite. Especially, the Method-II and Method-III gives a significant increase in the amount of submicron- size particles (<2µm). The smaller particle size observed especially in sample purified by method II and III is the affect of ion exchanges which reduces the particle size to colloidal clay particle range and also provide the advantages of making the highly dispersed suspension than calcium bentonite clay.
Moreover the small particle size of clay sample is one of the requirement of pharmaceutical industry, therefore it was concluded from the particle size analysis that the sample obtained by purification (Method-II and III) could be prefer because of their small particle size.

ii. **Group of Samples of Nowshera bentonite**

The data of Mastersizer for four samples from Nowshera site are presented here. In Fig. 4.15 the particle size distribution (PSD) plotted for the samples. It is observed that here the raw samples have broad PSD as compared to purified samples Method-I, II and
III centered at 5 µm and the largest size is about 30µm. After purification, by method-I and method-III (Soda Activated) the volume percentage of the submicron particle size increased drastically showing maxima at 1.7 µm and 2 µm respectively attribute to the clay particles, and the particle larger than 4µm became negligible as seen in Fig. 4.15 for these purified samples.

After NaCl treatment followed by sedimentation (Method-II), the plot showed bimodal size distribution, one maxima with smaller volume percentage centered at 0.23µm and other was higher percentage centered at 1.7µm. The shifting from large size to smaller size is attributed to the pure clay particles (fig. 4.15).

Fig. 4.15: Distribution of Particle size of Nowshera bentonite before and after purifications.
It was concluded from fig.4 that all the purification processes have increased the submicron particle volume percentage but it is not out of place to mention that method-II gives higher volume percentage of fine particles attribute to the negligible amount of impurities as compared to raw and other samples obtained by Method-I and Method-III.

iii. **Group of Samples of Peshawar bentonite**

The Peshawar bentonite sample in raw form showed very broad particle size distribution with two maxima one with relatively higher size volume percentage centered at 12µm and other at 4.5µm with asymmetric tail at the range of micron/submicron sizes (fig. 4.16). By the purification process (Method-I and III) the particle size distribution plot shifted to smaller particles size volume percentage with maxima centered at 1.8µm for both the sample but relatively higher volume percentage in sample purified by method-I, the tail of the curve of soda activated sample followed by sedimentation present the greater volume percentage of fine particle as compared to Method-I (fig. 4.16). The particle distribution plot of sample purified by Method-II also shifts to smaller size particles showing bimodal, one with higher volume percentage centered at 2.5µm attribute to the fine clay particle and other centered at 15µm shows agglomeration of clay mineral particles (fig. 4.16).
Fig. 4.16: Distribution of Particle size of Peshawar Bentonite before and after purifications.

However, the volume percentage of particle smaller than 1 µm is very small as compared to the sample purified by Method-I and Method-II but relatively greater than raw sample.

4.5 Thermo Gravimetric Analysis

Here the results of TGA study of studied bentonite clay sample are presented. The TGA results are presented as a plot of weight loss in percent against temperature as shown in fig. 4.17-4.19. TGA curves, displayed the stepwise weight loss in all samples.
The DTG plots are also presented in the same figures to indicate the endothermic peaks. The low temperature DTG endothermic peak in all the samples showed the weight loss mainly corresponds to the removal of remaining adsorbed liquid and interlayer water, while the higher temperature endothermic peak attributed to the dehydroxylation of silicate lattice. The TG curve of all the studied samples also showed the slight but continuous bleeding of weight loss between the temperature region of the hydration water loss and hydroxylation region.

i. **TGA/DTG Study of Karak Bentonite**

The TGA/DTG curves for raw and purified by methods I, II and III of Karak samples are presented in fig 4.17. According to the TGA/DTG curve, significant difference was observed in the thermal behavior among the purified and raw sample. The low temperature endothermic Peak at 79.7 °C shifted to higher temperatures in the sample purified by method-II to 89.73°C and Method-III to 94.83°C while decreased in Method-I to 72.03°C (Table-4.10). The appearance of second (shoulder) peak between ambient and 200°C in DTG curve of raw sample and the sample purified by Method-I was observed. This peak indicates the presence of divalent cations in the exchange layer of montmorillonite as this effect was reported by Charles for calcium bentonite [171]. After soda and sodium chloride treatment followed by sedimentation process (Method-II and III) the shoulder peak was found missing and the broadness of peak was also decreased. This is now clearly reflecting the replacement of Ca\(^{2+}\) cation by Na\(^{+}\) in the montmorillonite interlayer as reported by Charles for Sodium bentonite [171].
Fig. 4.17: TGA/DTG of Karak Bentonite before and after purification.

In all the purified samples the area of dehydroxylation endothermic peak slightly increased, which is showing the increase of weight loss and resultantly montmorillonite phase (Fig. 4.17, Table 4.10). The total weight loss was about 19% for raw sample. But in the samples purified by method I, II and III, it was 20%, 16% and 18% respectively. This variation in weight loss is associated with hydration water of exchangeable cations. The difference in temperature required to eliminate hydration water from the three purified samples was observed i.e. 143 °C, for samples purified by method-II and III, and 235 °C for the other purified sample (Method-I), this effect is related to the lower link energy of
the monovalent (Na\(^+\)) respect to divalent (Ca\(^{2+}\)) cation-water link as predicted by Visras and Lopez Galindo for other untreated calcium and sodium bentonite samples [21]. Hence the TGA/DTG results confirmed the conversion of calcium montmorillonite to sodium montmorillonite obtained by Method II, III, which is also consisting with the XRD and XRF results.

Table 4.10: TG/DTG data summary for Karak bentonite samples.

<table>
<thead>
<tr>
<th>Sample (Raw/Purified)</th>
<th>Wt loss (%)</th>
<th>DTG peaks temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25-105°C</td>
<td>105-240°C</td>
</tr>
<tr>
<td>Raw</td>
<td>10</td>
<td>5.3</td>
</tr>
<tr>
<td>Method-I</td>
<td>10.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Method-II</td>
<td>10.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Method-III</td>
<td>10.5</td>
<td>2.4</td>
</tr>
</tbody>
</table>
ii. **TGA/DTG Analysis for Nowshera Bentonite**

The four sample from Nowshera bentonite raw and purified by method-I, II and III were analyzed by TGA-60. The TG/DTG curves for the four samples of the Nowshera bentonite are plotted in fig.4.18.

![TGA/DTG curves](image)

**Fig. 4.18: TGA/DTG curves of Nowshera bentonite before and after purifications.**

Like Karak bentonite the shouldered peak was appeared in the curves for raw and sample purified by Method-I. This is again due to Ca$^{2+}$ cations. The shoulder peak was again found disappeared in samples purified by method-II and III. This is attributed to the NaCl and Na$_2$CO$_3$ treatment action in Method-II and Method-III respectively replacing Ca$^{2+}$ in the interlayer by (Na$^+$) and hence changing the Ca$^{2+}$-Montmorillonite to Na$^+$-montmorillonite. It was also observed that the temperature needed to liberate the
hydration water from raw sample and sample purified by method-I was high as compared to sample purified by method-II and III. This affect is directly related to The higher link energy of the divalent interlayer cations (Ca$^{2+}$) with water in raw and purified (Method-I) while lower link energy of Na$^{+}$ with water in sample purified by method-II and III.

The weight loss corresponds to dehydroxilation is seen to increase with purification process as reported in Table 4.11; this WAS because of the increase IN montmorillonite type of clay minerals with purification. Like the Karak bentonite the total weight loss of Nowshera raw sample (18.927%) and purified bentonite samples were not same but increased in sample purified by method-I from 18.927% to 21.356% and decreased to 16.680% in sample purified by method–II while it remaind constant in sample purified by Method-III as 18.882% (Fig4.18, Table 4.11).

**Table 4.11: TG data summary for Nowshera bentonite samples.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt loss (%)</th>
<th>DTG peaks</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22-105°C</td>
<td>105-240°C</td>
<td>240-540°C</td>
<td>540-900°C</td>
<td>Total</td>
<td>low</td>
</tr>
<tr>
<td>Raw</td>
<td>10.12</td>
<td>5</td>
<td>0.98</td>
<td>2.87</td>
<td>19</td>
<td>74.9</td>
</tr>
<tr>
<td>Method-I</td>
<td>11.5</td>
<td>5.5</td>
<td>1.08</td>
<td>3.34</td>
<td>21.4</td>
<td>86.5</td>
</tr>
<tr>
<td>Method-II</td>
<td>10.4</td>
<td>2.13</td>
<td>0.7</td>
<td>3.46</td>
<td>16.7</td>
<td>95</td>
</tr>
<tr>
<td>Method-III</td>
<td>11.7</td>
<td>2.274</td>
<td>0.9</td>
<td>4.015</td>
<td>18.8</td>
<td>93</td>
</tr>
</tbody>
</table>
iii. **TGA/DTG for Peshawar bentonite**

The TGA/DTG curves of four samples raw, purified by method-I, II and III of Peshawar bentonite are plotted in fig 4.19. In this case the lower temperature endothermic DTG peak correspond to weight loss due to hydration water was observed with shouldered peak for raw and sample purified by method-I. the shouldered peak was found disappeared in this lower temperature endothermic peak in the sample purified by method-II and III. Similar to Karak and Nowshera bentonite this again confirm the presence of Ca$^{+2}$ cations in the first two sample (raw and Purified by Method-I) and Na$^{+}$ cations in sample purified by method-II and III of Peshawar bentonite. The temperature required for liberating the hydration water from Peshawar bentonite was 230°C for raw sample and sample purified by method-I while 160°C samples purified by method-II and III.

![TGA/DTG curves of Peshawar bentonite before and after purifications.](image)

**Fig. 4.19:** TGA/DTG curves of Peshawar bentonite before and after purifications.
The temperature ranges 25-105°C, 105-240°C, 240-540°C and 540-900°C were chosen to describe the weight loss of raw and purified samples, summarized in Table 4.10. The weight loss correspond to dehydroxilation of silicate lattice increased in all the Peshawar purified samples but relatively more increased in sample purified by Method-III was observed as shown in fig. 4.19 and Table 4.12, which reflect to the decrease in impurities contents. Like Karak and Nowshera bentonite the change in total weight loss of Peshawar bentonite was observed after purifications process as shown in fig. 4.19 and Table 4.12.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt loss (%)</th>
<th>DTG peaks temperature in °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22-105°C</td>
<td>105-240°C</td>
</tr>
<tr>
<td>Raw</td>
<td>8.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Method-I</td>
<td>10.6</td>
<td>5</td>
</tr>
<tr>
<td>Method-II</td>
<td>9</td>
<td>2.6</td>
</tr>
<tr>
<td>Method-III</td>
<td>9.5</td>
<td>2</td>
</tr>
</tbody>
</table>

### 4.6 Morphological study

The morphological studies of all the samples were carried out through scanning electron microscope.
i. **SEM study of Karak Bentonite**

SEM studies were carried out to investigate the change in morphological features of natural and purified samples. In Fig. 4.20a and b the SEM micrographs of Karak raw bentonite are shown. The SEM image of the raw bentonite clay showed an aggregate of clay platelets of various sizes exfoliate by dispersion in water during sample preparation. They are arranged in face-to face patterns. Fig. 4.21a and b show the SEM photograph of the same Karak bentonite obtained after going through sedimentation process in aqueous suspensions to separate the nonclay minerals (method-I). The platelet structure is preserved. However, in this case the platelets nature of montmorillonite clay particles were clearly observed.

![SEM images of Karak Raw bentonite.](image)

Fig. 4. 20: SEM images of Karak Raw bentonite.
Fig. 4.21: SEM images of Karak bentonite purified by method-I.

It is clearly observed in fig. 4.22a, b and fig. 4.23a, b that the surface morphology of samples purified by method II and III were almost the same as original and the sample purified by method-I, but the only difference was that, the detected agglomeration of particle are relatively compact. It is probably due to the decrease of the basal distance after the treatment of original bentonite with NaCl and Na₂CO₃. The fine particles which had loosely aggregated together were also examined in Karak bentonite especially in sample purified by method-II.
Fig. 4. 22: SEM images of Karak bentonite purified by method-II.

Fig. 4. 23: SEM images of Karak bentonite purified by method-III.
ii. SEM Study of Nowshera Bentonite

The SEM study of Nowshera raw and purified samples are presented in fig. 4.24 to fig.4.27. The raw and purified sample of Method-I showed the same honeycombed structure of clay mineral flake(fig 4.24, fig 4.25). However, the structure of raw samples was little bit coarser because of the presence of impurities contents. In this case the particles are arranged in face to edge patterns. It is clear from fig. 4.26 that in case of sample purified by method-II the honeycombed structured little bite preserved but in case of sample purified by method III the morphology completely changed to planer structure (fig.4.27).

Fig. 4. 24: SEM images of Nowshera Raw bentonite.
That is probably happened due to occurrence of high tetrahedral and low octahedral isomorphous substitutions, which results from surface-surface interaction between the particles [127].

Fig. 4. 25: SEM images of Nowshera bentonite purified by method-I.

Fig. 4. 26: SEM images of Nowshera bentonite purified by method-II.
iii. SEM Study of Peshawar bentonite

The Peshawar clay has relatively different morphology from Nowshera and Karak raw bentonite (fig 4.28). Here the rounded masses of smectite were observed which may have formed from the pellet of volcanic ash. The SEM pictures of purified sample (Method-I) shows the same morphology as original sample but the structure was relatively dense as shown in fig. 4.29. Both original and purified sample (method-I) macroscopically has formed loose aggregated structure and the separate grains were composed mostly of parallel platelet particles arranged in face to face patterns. The SEM images of the samples purified by method-II and III are shown in fig 4.30 and 4.31 respectively. In these cases the same planer morphology was preserved; however the platy clay particles aggregates were little bite coarser especially in sample purified by method II. It is also observed in both the purified samples (method-II & III) that their
particles aggregates were relatively compact like Karak and Nowshera bentonites purified by the same procedure. This compact nature shows that the inter particle pores are relatively smaller in purified samples (Method-II & III) than original and the sample purified by method-I.

Fig. 4. 28: SEM images of Peshawar bentonite.

Fig. 4. 29: SEM images of Peshawar bentonite purified by method-I.
Fig. 4. 30: SEM images of Peshawar bentonite purified by method-II.

Fig. 4. 31: SEM images of Peshawar bentonite purified by method-III.
4.7 Surface area and pore size analysis

The adsorption-desorption isotherms of raw and purified samples of all three sites bentonites samples are shown in figure 4.32-4.34. Here P is the adsorption equilibrium pressure and $P_0$ is the saturation pressure (vapor pressure of the bulk liquid nitrogen at experimental temperature) of the adsorptive. The value of specific BET surface area, micropores volumes/area and pore size distribution obtained from N2 adsorption-desorption isotherms applying BET method, t-plot and BJH method respectively for all the samples before and after purification are summarized in Table-4.13, 4.14 and 4.15. According to the International Union of Pure and Applied Chemistry (IUPAC) classification of six type of recommended adsorption isotherm profile with four hysteries loop (H1, H2, H3, H4) [72], the observed isotherm profile were of type IV curve presenting an H4 hysteresis loop indicating a slit shaped porosity between plate like particles. The characteristic features of the hysteresis loop were due to the capillary condensation of liquid nitrogen taking place in slit like pores (mesopores). The part of the hysteresis below $p/p_0 \approx 0.45$ and the steeply sloped part in the relative pressure range of 0.98-1.00 are suggesting the presence of micropores and macropores respectively.

In addition to these common features of N2-adsorption-desoption isotherms for all the samples, the indigenous bentonites differ in characteristics which are described below.

i. Surface area and pore size analysis of Karak bentonite

The feature of all curves of Karak bentonite sample were the same, the only noticeable difference was the extension of Hysteresis loop by all the purification
processes indicating the increase in porosity and the higher adsorption volume than in the raw sample (fig. 4.32). As expected from the isotherms profile, the microspores volume represent 18-19% of the total porous volume of raw and all the three purified samples (Table-4.13). The surface area was not mainly external but also micro porous surface contributing up to 43% for raw, purified (Method-I, II) and 37% for purified (Method-III).

![Graph of Nitrogen adsorption-desorption Isotherms of Karak Bentonite before and after Purifications.](image)

**Fig. 4.32:** Nitrogen adsorption-desorption Isotherms of Karak Bentonite before and after Purifications.
Table 4.13: Surface area and porosity of Karak Raw and Purified samples.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Raw</th>
<th>Purified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method-I</td>
<td>Method-II</td>
</tr>
<tr>
<td>Specific surface area m²/g</td>
<td>75.2</td>
<td>95.29</td>
</tr>
<tr>
<td>Micropore volume cm³/g</td>
<td>0.014</td>
<td>0.018</td>
</tr>
<tr>
<td>Micropore area m²/g</td>
<td>32.49</td>
<td>41.28</td>
</tr>
<tr>
<td>External surface area m²/g</td>
<td>42.71</td>
<td>54.00</td>
</tr>
<tr>
<td>Total pore volume ( cm³/g) at P/P₀= 0.97</td>
<td>0.078</td>
<td>0.094</td>
</tr>
<tr>
<td>Average Pore Diameter (nm)</td>
<td>4.15</td>
<td>3.94</td>
</tr>
</tbody>
</table>

ii. Surface Area and Pore Size Analysis Nowshera Bentonite

In Nowshera bentonite the hysteresis loop of the entire purified sample overlapped and gave higher volume adsorption than in raw sample (fig. 4.33). The overlapping of isotherm of raw and purified sample approaching unity shows no change in the macropores volume by purification. Thus the adsorption volume increases due to increase in mesopores and micropores volume. The microspores volume represents 8% of the total porous volume of raw and increased to 13-14% for all the three purified samples (Table-4.14). Like Karak bentonite the micro porous surface contributing to the total surface area is about 31% in case of raw, 35% in case of purified (Method-I, III) and increase to 38% in sample purified by Method-II. The average pore size also decreased from 6.3 nm to 4.13 nm by the purification process.
Fig. 4.33: Nitrogen adsorption-desorption isotherms of Nowshera bentonite before and after purifications.

Table 4.14: Surface area and porosity of Nowshera raw and purified samples.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Raw</th>
<th>Purified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method-I</td>
</tr>
<tr>
<td>Specific surface area $m^2/g$</td>
<td>59.08</td>
<td>82.37</td>
</tr>
<tr>
<td>Micropore volume $cm^3/g$</td>
<td>0.008</td>
<td>0.013</td>
</tr>
<tr>
<td>Micropore area $m^2/g$</td>
<td>18.58</td>
<td>29.41</td>
</tr>
<tr>
<td>External surface area $m^2/g$</td>
<td>40.49</td>
<td>52.96</td>
</tr>
<tr>
<td>Total pore volume ( $cm^3/g$) at $P/P_0=0.97$</td>
<td>0.093</td>
<td>0.097</td>
</tr>
<tr>
<td>Average Pore Diameter (nm)</td>
<td>6.30</td>
<td>4.68</td>
</tr>
</tbody>
</table>
iii. **Surface Area and Pore Size Analysis of Peshawar bentonite**

In Peshawar bentonite the hysteresis loop of the entire purified samples also showed the higher volume adsorption in comparison to raw sample, the significant increase was observed in sample purified by Method-II and III (fig. 4.34).

![Nitrogen adsorption-desorption isotherms](image)

**Fig. 4.34: Nitrogen adsorption-desorption isotherms of Peshawar bentonite before and after purifications.**

The shape of adsorption-desorption branch of isotherms changed significantly; its slope remained steep in the relative pressure range of 0.98-1.00, which is suggesting
presence of macropores in the raw and purified samples. In the case of purified samples amount of plate like pores increased but this increase was dominant in the sample purified by Method II and III. The specific surface area and micropores volume/area was not considerably increased by purification but in case of sample purified by method-III significant increase was detected (Table 4.15). The total surface area in this case was also not mainly external but the microporous surface contributing up to 22% for raw, 33-36% for purified( Method-I, II) and increase to 40% for Purified (Method-III). The average pore size of Peshawar bentonite was also decreased from 6.7nm to 4.2nm by the purification processes.

The microspores volume represents 5% of the total porous volume of raw and increased to 11% in case of sample purified by Method-I, 15% for the remaining two samples purified by Method-II & III (Table 4.15).

**Table 4.15: Surface area and porosity of Peshawar Raw and Purified samples.**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Raw</th>
<th>Purified Method-I</th>
<th>Purified Method-II</th>
<th>Purified Method-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific surface area m²/g</td>
<td>47.3</td>
<td>93.83</td>
<td>94.58</td>
<td>128.45</td>
</tr>
<tr>
<td>Micropore volume cm³/g</td>
<td>0.004</td>
<td>0.014</td>
<td>0.015</td>
<td>0.023</td>
</tr>
<tr>
<td>Micropore area m²/g</td>
<td>10.43</td>
<td>31.51</td>
<td>34.75</td>
<td>52.16</td>
</tr>
<tr>
<td>External surface area m²/g</td>
<td>36.87</td>
<td>62.31</td>
<td>59.83</td>
<td>76.29</td>
</tr>
<tr>
<td>Total pore volume( cm³/g) at P/Po= 0.97</td>
<td>0.079</td>
<td>0.12</td>
<td>0.098</td>
<td>0.149</td>
</tr>
<tr>
<td>Average Pore Diameter (nm)</td>
<td>6.70</td>
<td>5.43</td>
<td>4.15</td>
<td>4.66</td>
</tr>
</tbody>
</table>
4.8 Microbiological Analysis

The microbiological studies for all raw and purified samples were carried out using the technique given in section 3.3.9. The results of microbiological studies for all raw and purified bentonites are stated in Table 4.16.

**Table 4.16. Microbiological results of raw and purified samples (CFU/g of sample).**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raw/Purified</th>
<th>E.Coli</th>
<th>Salmonella</th>
<th>Total Aerobic count</th>
<th>species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karak Bentonite</td>
<td>Raw</td>
<td>Absent</td>
<td>Absent</td>
<td>100-200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified (Method-I)</td>
<td>Absent</td>
<td>Absent</td>
<td>0-100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified (Method-II)</td>
<td>Absent</td>
<td>Absent</td>
<td>0-100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified (Method-III)</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Nowshera Bentonite</td>
<td>Raw</td>
<td>Absent</td>
<td>Absent</td>
<td>300-400</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified (Method-I)</td>
<td>Absent</td>
<td>Absent</td>
<td>100-200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified (Method-II)</td>
<td>Absent</td>
<td>Absent</td>
<td>0-100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified (Method-III)</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Peshawar Bentonite</td>
<td>Raw</td>
<td>Absent</td>
<td>Absent</td>
<td>100-200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified (Method-I)</td>
<td>Absent</td>
<td>Absent</td>
<td>0-100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified (Method-II)</td>
<td>Absent</td>
<td>Absent</td>
<td>0-100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified (Method-III)</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Pharmacopeias Bentonite/ Purified</td>
<td>Absent</td>
<td>--------</td>
<td>≤1000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The microbial test revealed that all the raw and purified samples were free of pathogen and the total bacteria count was also found within the permitted range of US pharmacopeia i.e. $< 10^3 \text{cfu/g}$ established for safe use of bentonite in the preparation of pharmaceutical products [25]. None of the sample was found to be contaminated by E.coli, and salmonella species (Table 4.16). Hence the absence of E.coli requirement of pharmacopeias was also fulfilled by the studied bentonites (US Pharmacopoeia, 2007a) [25]. On these test results basis the studied bentonite in raw and purified form was found microbiologically innocent and could be used safely for pharmaceutical purposes.

### 4.9 Pharmacopeias requirements

All the samples from the three sites of local bentonite mines are studied for its pharmacopeias suspension tests (i.e. swelling volume, sedimentation volume or gel formation and pH value) required to assist the suitability of these bentonites for specific pharmaceutical uses and the study is presented for each sample separately. These tests were carried out according to the techniques given in section 3.3.10

#### i. Bentonite samples from Karak site

The pH value, gel formation or sediment volume and swelling volume of Karak samples are shown in Table 4.17. The pH values of 4% aqueous dispersions of Karak samples were found in the range of 8.33 to 10.5. The pharmacopeias permitted pH value is 8.5 to 10.5. The pH value for raw samples is near but not within the specific range of major pharmacopeias. For gel formation or sedimentation volume measurement, the suspension was prepared by mixing 3 g clay in 100 ml distilled water using high speed
mixer at 1000 RPM for 20 min and then was kept undisturbed for 24 h. After 24 h the clear supernatant was quantified as gel formation value. The gel formation value of raw and sample purified by method-I was <2 mL and for sample purified by method II and III was <1 mL (Table 4.17, fig 4.36). These results for gel formation indicate that Karak bentonite in raw as well as purified form fulfilled the pharmacopeias requirement i.e. ≤ 2mL.

The swelling property was determined by adding 2g powder bentonite sample in gradually to 100ml distilled water contained in a cylinder of 100ml capacity and the apparent volume was quantified after 2 h. The apparent volume was termed as swelling capacity of the samples. For raw sample it was 7ml as shown in Table 4.17 and in fig 4.35. The swelling power of the Karak bentonite was observed to increase to 11mL, 34mL and 30 mL purified by method-I, method-II and method-III respectively. The swelling property results revealed that only the sample purified by method-II and Method-III were within the pharmacopeia requirement limit while the raw and sample purified by method-I were found outside the minimum pharmacopeias limits i.e ≤ 22 mL.

The increase in swelling capacity of sample purified by Method-II & III indicates that predominantly Ca$^{2+}$ cations have been replaced by Na$^+$ ion. The logic behind the increase in swelling property by these two methods (i.e. Method-II & III) can be explained as follow.
From the study of XRD, DTG and XRF results it has been confirmed that the Karak bentonite containing mainly montmorillonite with Ca\(^{2+}\) exchangeable cations. The strong electrostatic attraction between this Ca\(^{2+}\) cations and the negatively charged clay surfaces make the unit layers closely spaced, and thus the interlayer hydration is limited to two or three molecular layers of water. On other hand electrostatic attractions between the exchangeable cation (Na\(^{+}\)) and the clay layers are much weaker as compared to Ca\(^{2+}\) cations in Ca\(^{2+}\)-montmorillonite. This weak electrostatic interaction forms a loose and
dispersed structure of particles. As a result the excess water enter the interlayer spaces, surrounding Na\(^+\) ions with a larger hydration shell and accommodate a large portion of water in the system [172-173].

![Images showing sedimentation volume of Karak sample purified by (a) Method-I (b) Method-III (c) Method-II, and (d) Raw bentonite.](image)

**Fig. 4. 36: Images showing sedimentation volume of Karak sample purified by (a) Method-I (b) Method-III (c) Method-II, and (d) Raw bentonite.**

On the basis of these pharmacopeia suspension tests it was concluded that in specific pharmaceutical applications such as suspending agent and disintegrate agent, the sample purified by method-II and III should be preferred because of high swelling power and gel formation.
Table 4.17: The numerical values of pH, Swelling properties and gel formation of Karak bentonite.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Raw</th>
<th>Purified Method-I</th>
<th>Purified Method-II</th>
<th>Purified Method-III</th>
<th>Pharmacopeias Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH Value (4g/200mL)</td>
<td>8.33</td>
<td>8.5</td>
<td>9.7</td>
<td>10.5</td>
<td>9-10.5</td>
</tr>
<tr>
<td>Swelling Property (2g/100mL)</td>
<td>7 mL</td>
<td>11 mL</td>
<td>34 mL</td>
<td>30 mL</td>
<td>≤ 22 mL</td>
</tr>
<tr>
<td>Gel formation (6g/200mL)</td>
<td>&lt;2 mL</td>
<td>&lt;2 mL</td>
<td>&lt;1 mL</td>
<td>&lt;1 mL</td>
<td>≤ 2 mL</td>
</tr>
</tbody>
</table>

ii. Bentonite samples from Nowshera site

The swelling capacity, sedimentation volume or gel formation and pH values of Nowshera bentonite samples before and after purification by three different methods are presented in Table 4.18. The pH value of all the samples of Nowshera bentonite except raw bentonite meets the pharmacopeia requirements, with the pH values between 8.5 and 10.5.

The sediment volume of the raw and purified samples was observed within the permitted range of pharmacopeias limit i.e. ≤ 2 mL (fig 4.38a-d). The swelling volume of raw sample was 8 mL. This swelling property of Nowshera bentonite was improved with the purification process and the high value of swelling of 34 mL and 30 mL were observed for the samples purified by method-II and III (fig. 4.37a-d). This increase in swelling capacity of the sample purified by Method-II & III again confirmed the replacement of Ca$^{2+}$ by Na$^{+}$ cation as in discussed in Karak bentonite.
Fig. 4.37: Images showing swelling volume of Nowshera (a) raw bentonite, and samples purified by (b) Method-I (c) Method-III (d) Method-II.

Fig. 4.38: Images showing sedimentation volume of Nowshera samples purified by (a) Method-I (b) Method-III (c) Method-II, and (d) raw bentonite.
Table 4. 18: The numerical values of pH, Swelling properties and gel formation of Nowshera bentonite.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Raw</th>
<th>Purified</th>
<th>Pharmacopeia Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Purified</td>
<td>Pharmacopeia Requirements</td>
</tr>
<tr>
<td>PH Value (4g/200mL)</td>
<td>7.82</td>
<td>8.82</td>
<td>8.7</td>
</tr>
<tr>
<td>Swelling Property (2g/100mL)</td>
<td>8mL</td>
<td>14mL</td>
<td>37mL</td>
</tr>
<tr>
<td>Gel formation (6g/200mL)</td>
<td>2mL</td>
<td>&lt;2mL</td>
<td>1mL</td>
</tr>
</tbody>
</table>

iii. Bentonite Samples from Peshawar Site

The swelling property, gel formation and pH values of Peshawar bentonite before and after purification are shown in Table 4.19. The pH value of all the samples of Peshawar bentonite meets the pharmacopeia requirements. The sedimentation volume or gel formation of Peshawar raw bentonite was 4ml which is not fell within the permitted range of pharmacopeia requirement i.e. ≤2mL.
This gelling property improved by purification process and fulfilled the pharmacopeia requirement (Fig. 4.39). The swelling property of Peshawar bentonite before purification was 4ml. This value was slightly improved by purification and the maximum value of 14mL and 15mL were obtained by Method-II and III respectively (fig. 4.40), which was less than the minimum required swelling power of 20-24mL to be used as specific pharmaceutical product such as suspension.
Table 4. 19: The numerical values of pH, Swelling properties and gel formation of Peshawar bentonite.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Raw Method-I</th>
<th>Raw Method-II</th>
<th>Purified Method-I</th>
<th>Purified Method-II</th>
<th>Purified Method-III</th>
<th>Pharmcaceopia Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH Value (4g/200mL)</td>
<td>8.8</td>
<td>9.4</td>
<td>9.58</td>
<td>10.3</td>
<td>9-10.5</td>
<td></td>
</tr>
<tr>
<td>Swelling Property (2g/100mL)</td>
<td>4mL</td>
<td>5mL</td>
<td>14mL</td>
<td>15mL</td>
<td>≤ 22 mL</td>
<td></td>
</tr>
<tr>
<td>Gel formation (6g/200mL)</td>
<td>4mL</td>
<td>&lt;2mL</td>
<td>&lt;2mL</td>
<td>&lt;2mL</td>
<td>&lt;2mL</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4.40: Images showing swelling volume of Peshawar (a) raw bentonite, and samples purified by (b) Method-I (c) Method-III (d) Method-II.
CONCLUSION

As we discussed in chapter-II, the bentonite is the most used clay in various industry. Bentonites are natural material varies in composition and texture; hence they are not same and not equally well in performance for specific industrial use. They can only be consider as raw material for specific industry that once evaluated and/or purified, modified to fulfill the basic requirement and may achieve the status of industrially suitable substance. This suitability study for a particular industry requires a lot of work to compile research data on industrial properties and modification technique. Therefore this study was limited to evaluate the pharmaceutical suitability of bentonite. The mineralogical, chemical, microbiological and physicochemical studies are the main requirement to evaluate bentonite for pharmaceutical purpose. Though in Pakistan, bentonites are found in abundant but still they have not been investigated for pharmaceutical applications. Therefore, it was very important to characterize Pakistani bentonite for different pharmaceutical purposes in order to support the local pharmaceutical industry and add value to convert it into pharmaceutical product. So to explore the potential use of local bentonite in pharmaceutical sector, three bentonite samples were selected from three different Pakistani sites. The raw samples were processed, purified by different methods and tested for its suitability for pharmaceutical purposes. The characterization report of all samples collected for this research work has been discussed and presented in chapter-4. The following conclusion is derived.

The mineralogical study reveals that all the three samples are mainly composed of di-octahedral smectite mineral called montmorillonite. The results also show that all the
studied bentonite samples are calcium bentonite containing not only montmorillonite but also small amounts of illite and quartz. In addition, kaolinite in Karak bentonite, albite in Peshawar and Nowshera bentonites were also observed. The quantitative XRD analysis confirmed the mineralogical variation in all the three raw samples. The Karak bentonite was found rich in clay content as compared to Peshawar and Nowshera raw bentonite samples.

The differences in the major elemental composition in all the raw samples were also detected. After purification by three methods the elemental composition were significantly affected in all the three studied samples showing the removal of impurities. The chemical composition, particularly trace elemental content such as toxic elements i.e. Pb and As, microbial content were inside the limit of pharmacopeia requirements for all raw and purified samples.

Although all the studied raw samples were found innocent chemically and microbiologically but minralogically toxic as they have quartz, which is harmful to the human health for long use. The raw bentonites also failed to comply with the pharmacopeia requirements regarding the swelling (for all raw samples) and gel formation (only for Peshawar sample) which are very important for specific pharmaceutical use such as oral and topical suspension and tablet disintegrant agent. Therefore the purification of all these samples was necessary before their use in pharmacy.

All the raw bentonite samples were made mineralogically innocent and also its related properties such as swelling volume and gel formation were improved by three
different purification methods. It is interesting to note that the calcium bentonite was also successfully converted to sodium bentonite by the purification methods II and III for all samples confirmed by the XRD, XRF and DTG results. All the purified samples especially the samples purified by methods II and III, have a promising swelling, gelling and other related properties required for pharmaceutical use. The experimental analyses confirm that, the purification processes make all the bentonite clay samples from the three sites of Pakistan suitable for pharmaceutical application particularly pharmaceutical excipient. The high CEC and large specific surface area for all purified samples were found which suggesting their use as suitable raw materials in the formulation of controlled drug released system which is presently one of the most emerging fields of bentonite application.

All the purified samples could also be used for the formulation of oral suspension and as a tablets disintegrant agent because of its high swelling volume and gel formation or sedimentation volume. The interlayer cations, chemical composition, colour difference, small particle size and other studied properties were found different for all the purified samples which obviously give different behavior while using it for pharmaceutical and cosmetic use.

The possibility as disintegrating agent in tablet formulation depends on the swelling capacity as previously studied [56-58]. Thus for excellent use the sample purified by method-II and III especially Karak and Nowshera samples could be preferred because of their high swelling capacity and gelling properties.
The high CEC and pore size distribution of purified samples ensure their use as drug adsorbent to delay drug release. Difference in pore size distribution and CEC of the purified samples may give different performance for supporting drugs as already proved for similar material [174].

The difference in surface area and pore size distribution in the purified samples could testify changes in adsorption capacity, as previously studied this effect for bentonite by Bejmeular et al. [175].

As the purified bentonites vary in their interlayer cations, CEC, this variation could result in changes in drug loading ability or adsorption capacity as studied already [53]. The purified bentonite particularly sample purified by method-II and III could preferably be used as suspending agent because of its greater sedimentation volume and high swelling capacity, as recently reported by Lopez-Galindo et al. [14].

It is not out of place to mention that, the entire sample obtained by soda activation at different ratio are not only found mineralogically pure but also the swelling power is improved and fulfilling the minimum swelling volume requirement for pharmacopeias i.e. ≥24 mL. Thus not only sodium bentonite obtained by soda activation at 3% i.e. P3 and 5% activation dose (K5,N5) but also the sample (K2, K3, N2, N3 and P2) obtained by other ratio of sodium carbonate may be used for specific Pharmaceutical use. The CEC of soda activated samples varies in magnitude, which may show different performance in drug loading ability in control drug release system as studied for bentonite [53], and proved for similar materials [174].
RECOMMENDATIONS FOR FUTURE WORK

In the present study the pharmaceutical suitability of indigenous bentonite clay were investigated. It might be of interest to study these bentonites for specific pharmaceutical purposes especially for drug delivery system as their high CEC and high specific surface area suggesting their use for the said purpose.

It is also recommended to study the other specific properties related to particular industrial use i.e. Foundries, mineral oil industry, building industry, paper industry etc in order to add the high added value to our local bentonite.

It will also be of interest to study the effect of other inorganic chemical treatment such as H$_2$SO$_4$, NaOH and HCl etc on our local bentonite.

The studied Pakistani bentonite have high contents of montmorillonite with high Cation Exchange Capacity which suggesting their use for synthesising organoclay. Therefore it might be of interest to use these bentonite especially Karak and Nowshera bentonite for synthesis of organoclay which have wide spread industrial application including the area of health.
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