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A NEW APPROACH OF EGYPTIAN CALCIUM CARBONATE UTILIZATION AS INGREDIENTS OF TOOTHPASTE MANUFACTURE

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ABSTRACT

Nowadays, the calcium carbonate material is used on a wide scale as an effective dietary calcium supplement, antacid, phosphate binder, and base material for medicine tablets such as baking powder and toothpaste components. This study introduces a contribution and characterization of the Beni Khaled calcium carbonate for toothpaste pharmaceutical ingredients. Calcium carbonate samples were ground to very fine grain size ranging from 5 - 20 um to be used as an abrasive ingredient of toothpaste components by 25 - 55 % as per international standard. The testing techniques include measuring of physical, chemical and biological properties; XRF, XRD, water absorption, matter soluble in water, and biological study of harmful bacteria types which disappeared with the heating of $CaCO_3$ powder up to 121ºC.

Keywords: Calcium Carbonate; pharmaceutical filler; Minia; toothpaste; biological properties.

INTRODUCTION

The main ingredients of toothpaste composed of abrasive and binders. The most ore mineral used in toothpaste abrasives are (1) Ground calcium carbonates (GCC) and precipitated calcium carbonates (PCC) with a ratio of up to 55%. The size of $CaCO_3$ utilized in different kinds of toothpaste varies from 0.7 to 10 um. (2)Silica is composed of quartz or silicon dioxide. (3) Mica used as a mild abrasive to give the polishing of the tooth appearance. (4) Titanium is a hard silvery-white metallic element that is found in the ilmenite minerals. Each abrasive also has slightly different cleaning properties, and a combination of them might be used in the final product as per Burnet et al. (2006)**.**

In the present study the main target to assess the calcium carbonate properties to be utilized in toothpaste as abrasive ingredients. This study carries out the exploration of high-quality chalk and limestone from open cast mines and chooses the samples with a high content (> 90% $CaCO_3$) and about 95 % whiteness at Beni Khaled area according to Gaber (2013). The large grain size introduced for crushing to obtain a small size, and then to the processing plant for more crushing by using jaw or cone crusher up to 4 cm, and finally more process of grinding to produce a very fine CaCO3 powder with 10 um size.

MATERIALS AND METHODS

Twenty calcium carbonate samples were collected from Beni Khalid "Minia". It is located between latitudes 28° and 28°40'N and longitudes 30° 50' and 31° 30' E. The exposed calcium carbonate rock of Minia – Maghagha areas include five units of Middle Eocene age and composed mainly of calcium carbonate as per Abdel Tawab (1994) & Abu El Ghar et al. (2005). The collected samples were ground to very fine grain size ranging from 5 – 20 um to determine the physical, chemical, and biological properties (Fig.1-b).

The assessment of ground calcium carbonate characterizations was conducted as per Indian standard 918 (2006), and the following quality control testing of GCC "Physical properties, XRD and XRF, and biological testing Counts" was conducted as follows:

a b

Fig. 1: Field photos for a quarry of pure limestone at El Minia, East Nile Valley, (a) Large limestone outcrop (b) Calcium carbonate powder (5-20um

Microbial isolation

Bacterial isolation

By using 1gm of each $CaCO_3$ sample was merged in 9 ml of sterilized saline solution, shacked for about 5 min. and serial dilution was made. Different types of enrichment and selective media were used as follows; Plate count agar media (PCA) as per Atlas (2004), Nutrient agar media as per Atlas (1993) & Cowan (1993), Mannitol salt agar media (MSA) as per Idziak and Mossel (1980) and Blood agar media as per Reddy et al. (2007).

Fungal isolation

The Isolation was carried out as follows; Malt extracts agar media as per Gutz and Doe (1973).

Bacterial identification

Morphological studies

Morphological characters of all pure bacterial colonies including, pattern, color, and arrangement on the agar plates, were recorded.

Staining methods

a- Simple stain was prepared and stained according to Curry et al. (1993), using methylene blue and crystal violet.

b- Gram-stains were prepared using a modification of Gram stain as per Hucker (1927).

c- Catalase property was tested for the obtained species as per Benson (2001).

Fungal Identification

Fungal genera and/or species were identified morphologically on the plate agar media and by microscopic examination.

Sterilization of the $CaCO_3$ samples

It was sterilized by wet sterilization using Autoclave at two levels of temperature as follows:

Autoclaving at 110°C for 15 min

One gram of each $CaCO_3$ samples was merged in 9 ml of sterilized saline solution, shacked for about 5 min. and serial dilution was made and placed in autoclave adjusted at 110°C for 15 min. After sterilization, all the above media were inoculated from the serial dilutions, incubation for each media at 110°C and 15 minutes then the resultswere recorded.

Autoclaving at 121°C for 15 min.

One gram of each CaCO3 sample was merged in 9 ml of sterilized saline solution shacked for about 5 min. and serial dilution was carried out and placed in an autoclave that adjusted at 121°C for 15 min. Different types of enrichment and selective media were inoculated from the serial dilutions and incubations were conducted at 121°C for 15 minutes.

RESULTS AND DISCUSSION

The methodology included the agar diffusion technique that has been used as a regular method of checking the antibacterial sensitivity. Although this method is convenient for fluid materials like water, it's been also used for antimicrobial assessment of semi-solid matters which are fluid in the presence of saliva or water such as toothpaste.

Toothpaste (dentifrice) is important for daily oral hygiene routine. The most target of toothpaste to improves the mechanical brushing and cleaning of teeth. The function of oral hygiene using toothpaste is to cut back oral bacterial flora and deliver fluoride to the teeth. The fluoride plays a crucial role to shield teeth against attack of bacteria, it helps remove plaque, prevent decay by strengthening enamel, found naturally in many everyday things including food and water. The carbonate within the Pharmaceutical industry is employed as an abrasive ingredient of toothpaste and cosmetic industry and structure to 55% as per Kiruthiga (2011).

The physic-chemical analyses were conducted to assessment the Egyptian pure calcium carbonate located in Mina area as follows:

Physical properties were accomplished for Beni Khaled calcium carbonate powder as follows: *Loss on drying*: Weigh 5 g of the fabric in a very weighed, clean and dry squat form weighing bottle, and dry to constant weight at 105°C ± 2'C.

Loss on drying, per cent by mass =100 x W_l/W_2

Where; W1 wt. of sample before heating and W_2 wt. of sample after heating to 105°C)

The results of calcium carbonate powder loss on drying is ranging from 0.05 – 0.07, these values comply with the desired parameter as indicated in Table (1), and thought of low percentage and good property for toothpaste as per American Standard for Testing Material - D 280 (1987).

Specific gravity: The studied samples of calcium carbonate were measured as per American Standard for Testing Material D- 153 (1989), and the results are 2.65 g/cm³ as shown in Table (1).

Table 1: Physical and chemical properties of studied calcium carbonate

Physical Properties	Result	**Chemical Properties**	Result
Whiteness	96 %	CaO	55.65 %
Water Absorption	40 to 50 ml/100 gm	MgO	0.10 %
Oil Absorption	30 to 32 ml/100 gm	L.O.I	43.75 %
Loss on Drying at 105° C	0.05 – 0.07%	Iron as Fe	0.11 ppm
Specific Gravity	2.65 g/cm³	Matter insoluble in HCL	1 %
Moisture %	0.05 to 0.07	pH	8.5
Hardness (*Moh*'s)	3	Phosphate	150 ppm
Matter soluble in water	0.75 %	Chloride as Cl	0.18 ppm
Acid soluble	99 %		
Average Particle Size	5 to 20 µm		

Matter soluble in water: The carbonate powder shall be insoluble in water as per American Standard for Testing Material D- 2198 (1989), except some traces of soluble salt. The result of studied samples of soluble matter is 0.75 % as indicated in the Table (1),

Oil absorption: Administrated by using linseed oil which mixed with $CaCO_3$ powder up to obtain the paste as per American Standard for Testing Material D- 234 (1991) and American Standard for Testing

Material D-281 (1989), and then a quantity of oil was measured and located in the range of 30 to 32 g/100g.

Particle size "Fineness": The studied samples were ground to small size about 10 um using ball mill (Fig. 2). The particle size curve show that the studied samples are ranging from 5- 20 um as illustrated in (Fig. 3), whereas the 80% of the studied sample is smaller than 20 um grain size.

Whiteness (5GE): The $CaCO_3$ was examined by using Dr Lange's equipment, and also the results verify the degree of whiteness value 96% as mentioned in Table (1).

Fig.2. Ball mill for grinding of limestone

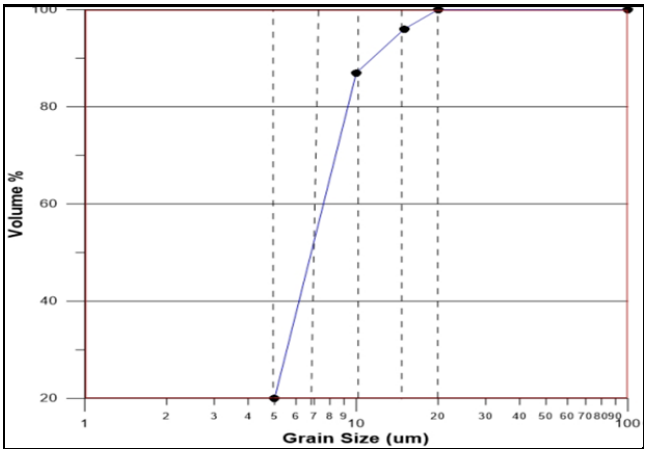

Fig. 3. $CaCO_3$ powder grain size (5-20 um)

Chemical analysis

- *X-Ray Diffraction*: The carbonate per cent within the examined samples are starting from 99.30 to 99.55 %, which classified as high purity carbonate as per Harrison (1992).
- *XRD* of representative samples from Beni Khaled showing that the calcite mineral is the dominant mineral in carbonate rocks with a mean ratio of 99.55% as illustrated in (Fig.4).
- *X-Ray Florescence*: It's administrated to 4 samples reflects that the most elements is CaO 55.65; which implies that the ratio of $CaCO_3$ is 99.55, which comply with the specification of $CaCO_3$ required for toothpaste. These results indicate that the studied sample possesses high purity and suitable for toothpaste manufacture.
- *pH value*: The hydrogen ion concentration test was completed for the collected sample as per American Standard for Testing Material - E 70 (1990), to work out the worth of alkaline or acidity of $CaCO_3$ powder and also the results of pH gives 8.5 as illustrated in Table (1).

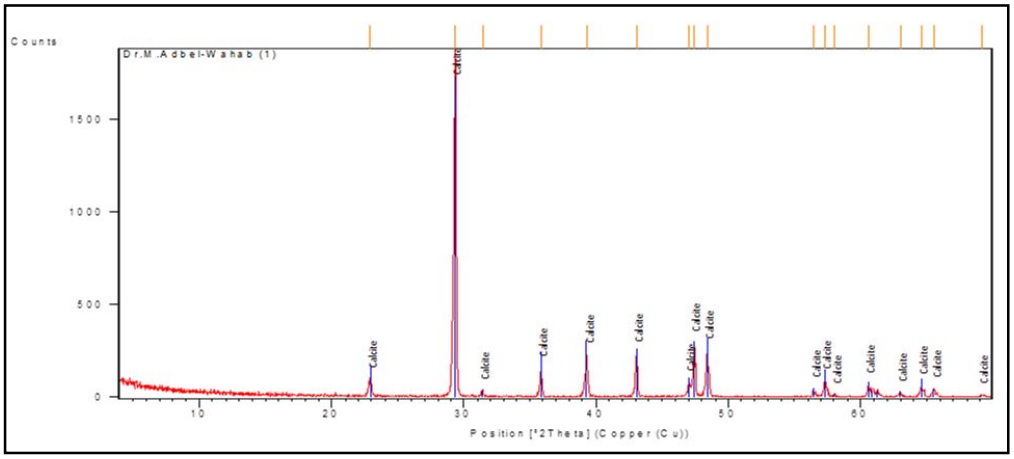

Fig. 4: XRD of Limestone at Beni Khaled area.

Biological testing

Isolation and identification of bacteria from calcium carbonate

This test was completed to isolate and identify the various bacterial species that occurred within the different carbonate samples. Different types of enrichment and selective media were used for counting and isolation of bacterial species that occurred within the carbonate of the Beni Khaled area. The results indicate that 1 gm from each carbonate sample contains two types of bacterial species as recorded within the Table (2 & 3).

Table 2: Characters of different bacterial Genera and /or Species found in the studied $CaCO_3$ sample 1

Test	Result	
	Species (1)	Species (2)
Plate count agar media	22 $x10^6$	4 $x10^6$.
Nutrient agar media	Small colonies with yellow color.	Large colonies with white color.
Mannitol salt agar media	Growth with yellow color zone	-ve
Blood agar media	β - heamolysis	γ -heamolysis
Simple stain	Staphylococci shape	Streptobacilli shape
Gram stain	+ve	-ve
Catalase activity	+ve	+ve

Table 3: Characters of different bacterial Genera and /or Species found in the studied $CaCO_3$ of sample 2

Test	Result	
	Species (1)	Species (2)
Plate count agar media	13 $x10^6$.	45 $x10^6$.
Nutrient agar media	Small colonies with yellow color.	Large and yellow colonies.
Mannitol salt agar media	Growth with yellow color around the colonies	-ve
Blood agar media	β - heamolysis	γ -heamolysis
Simple stain	Staphylococci shape	streptobacilli shape
Gram stain	+ve	-ve
Catalase activity	+ve	+ve

Isolation and identification of fungal species of studied carbonate

Malt extract agar media were used for the isolation of various fungal species that occurred in samples. From the morphological and microscopic examination, Table (4) shows three differing kinds of Fungi observed in carbonate sample 1; white with black spots *Rhizopus* 1x104, yellow *Penicillium* 2x104, and white *Aspergillus* 4x 104. Table (5) indicates the presence of 4 kinds of fungi in carbonate 2; yellow *Penicillium* 3x 104, white *Aspergillus* 3x 104, deep yellow *Aspergillus* 2x 104, grey *Aspergillus* 4x 104.

Table.4: Characters of fungal Genera found in the studied calcium carbonate sample 1

Three different types of fungi were observed			
	Color	**Total No.**	**Microscopic examination**
Species (1)	White with some black parts	1 x 10^4	Rhizopus shape
Species (2)	Yellow	2 x 10^4	Penicillium shape
Species (3)	White	4 x 10^4	Aspergillus shape

The results illustrated within the Table (6) shows the odds of microorganism types detected within the $CaCO_3$ sample 1 is *Streptobacillus* with 22 – 84 %, *Staphylococcus* with 15 – 74 %. In $CaCO_3$ sample 2 the microorganism found *Penicillium* with 25 – 28 %, *Aspergillus* with 57 – 75 %, *Rhizopus* with the percentage starting from 0 to 14%.

Table 5: Characters of fungal Genera and /or Species found in the studied calcium carbonate of sample 2

Four different types of fungi were observed			
Species (1)	Yellow	3×10^4	Penicillium shape
Species (2)	White	3×10^4	Aspergillus shape
Species (3)	Deep yellow	2×10^4	Aspergillus shape
Species (4)	Gray	4×10^4	Aspergillus shape

Table 6: Relative percentages of different microorganism types detected in the studied $CaCO_3$

Types of bacterial species in sample 1	
Species	**Percentage (%)**
Streptobacillus	22 – 84 %
Staphylococcus	15 – 77 %
Types of fungal species in sample 2	
Species	**Percentage (%)**
Penicillium	25 – 28 %
Aspergillus	57 – 75 %
Rhizopus	0 – 14 %

Sterilization of the studied calcium carbonate

Autoclaving at 110 °C for 15 min

On plate count, agar media (9×10^4) and (4×10^2) of *Streptobacilli* species were found in 1gm from sample1 and 1gm of sample 2, respectively, while all other microorganisms were killed. There is no growth of microorganisms was observed on mannitol salt agar media or blood agar media. This indicates that the *Staphylococcus* and *Fungal* species were killed at 110ºC and 15 minutes of sterilization.

Autoclaving at 121 °C for 15 min:

The Calcium carbonate powder of Beni Khaled area sterilization carried out on 121°C for 15 min, and then the observation indicates that is no growth of bacterial or fungal species on any type of enrichment and selective media, this result confirms the killing of all microorganisms. The study recommends sterilizing the calcium carbonate at 110ºC for 15 minutes before using in pharmaceutical or medical industries to ensure there are no hazardous microorganisms.

CONCLUSIONS

This study indicates the following positive conclusions:

1- Biological study at ambient temperature indicates the presence of some microorganisms.

2- Biological study and sterilization at 121°C and 15 minutes show no presence of harmful microorganisms and can be used safely in producing pharmaceutical products.

3- Cooperation of the geologists and pharmacists to find the Egyptian substitute ores instead of imported ones and safe hard currency.

4-The presence of a lot of reserve of $CaCO_3$ in the Beni Khaled area can be mined by an open cast and located near infrastructure road and El Mina industrial area.

5- XRD analysis indicates that the Calcium carbonate samples have a high content of $CaCO_3$ "99.55%" suitable for the pharmaceutical industry.

Conflict of Interest: The authors have declared no conflict of interest.

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نهج جديد لاستخدام كربونات الكالسيوم المحلية فى صناعة معجون الأسنان

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الخلاصة

يتم استخدام مادة كربونات الكالسيوم ، في الوقت الحاضر، على نطاق واسع كمكملات الكالسيوم الغذائية الفعالة، مضادات الحموضة، مواد رابطه الفوسفات ، والمواد الأساسية المكونة للأقراص الطبية مثل باكينغ باودر وكذلك فى معجون الأسنان و هو موضوع الدراسة .

تقدم هذه الدراسة مساهمة فى توصيف كربونات الكالسيوم الموجودة بكثرة فى منطقه بني خالد بالمنيا لتصنيع المكونات الصيدلانية لمعجون الأسنان الذى يستخدم على نطاق هائل للإنسان.

تم تجميع عينات كربونات الكالسيوم من منطقه الدراسه و كذلك تم طحن هذة العينات الى حبيبات دقيقة جدًا يتراوح حجمها بين ٥ إلى ٢٠ ميكرون و تم إجراء التجارب المعملية للوقوف على مدى ملائمتها للإستخدام كعنصر كاشط فى مكونات معجون الأسنان حيث تمثل نسبه كربونات الكالسيوم قدرا كبيرا من مكونات معجون الأسنان حسب كل نوع و تتراوح من ٢٥ إلى ٥٥٪ وفقًا للمعايير الدولية .

وتشتمل تقنيات تقييم العينات على قياس الخصائص الفيزيائية والكيميائية والبيولوجية ؛ و XRF و XRD وامتصاص المياه ، والمواد القابلة للذوبان في الماء ، كما شملت الدراسة البيولوجية التعرف على أنواع البكتيريا الضارة الموجوده فى كربونات الكالسيوم الطبيعيه .

أظهرت النتائج التى تم الحصول عليها للعينات مطابقه الخصائص الفيزيائيه و الكيميائيه لمواصفات كربونات الكالسيوم المستخدمه فى صناعه معجون الأسنان .

و قد عالجت الدراسه كيفيه التخلص من هذة البكتريا بتسخين مسحوق $CaCO_3$ حتى ١٢١ درجة مئوية و تم القياس مرة أخرى ووجد إختفاء جميع أنواع البكتريا وبذلك تسلط الدراسه الضوء على إستخدام جديد لكربونات الكالسيوم المحليه فى تصنيع نوعيات من معجون الأسنان و الذى أصبح لاغنى عنه لجميع أفراد الأسرة للعنايه باللثه و الأسنان . كما يساعد هذا البحث فى زيادة القيمه المضافه للخامات المحليه و هذا ما تسعى اليه الدوله المصريه فى الوقت الحالي.