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Antibacterial and antifungal studies of Zn-CaB as a promising antimicrobial agent incorporated health care and cosmetic products

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The Zinc doped calcium borate (Zn-CaB) was synthesized by the co-precipitation method. Zinc was known to exhibit a good antimicrobial effect without causing any environmental issues. However, the antimicrobial activity of zinc is relatively low as compared to silver or copper analogues. In the present investigation, an attempt was made to incorporate zinc in the matrix of calcium borates to enhance the antibacterial effect. Cosmetic products were made using the zinc-calcium-borates and the antibacterial/antifungal activity was evaluated and compared with the commercial products. The studies revealed that the antibacterial activity of Zn-CaB was equivalent to that commercially available product with a maximum of 99% reduction in bacterial strains and above 95% reduction in fungal strains. An attempt was made to incorporate a bacteriostatic effect on fabric by adding zinc-calcium-borates to detergent which exhibited prolonged antibacterial activity, even after 3 washing cycles. The cosmetic products were also evaluated for their thermal and photostability.

1. Introduction

Infectious diseases are a major threat to humanity as the death rate has been increased by the infectious microorganisms in the developing and under-developing countries every year. The development of novel antibacterial agents is the only way to subside infectious diseases. The antibacterial agents find major applications in pharmaceutical, medicine, water treatment, hygiene products, and food packaging and textile industries.[1-4] Several inorganic and organic compounds have been employed as antimicrobial agents in controlling infectious diseases. Generally, the microorganism slowly develops resistance against the organic antimicrobial agents. The inorganic antibacterial agents like Ag, Zn and Cu nanoparticles can be leached into the environment which causes an environmental issue besides their excellent antimicrobial activity. The above-said problems can be outwitted by incorporating the metal ions into calcium borates. Calcium borates have been selected to prepare the boron-based antibacterial agents owing to their thermal stability, non-toxic and non-reactive nature.

Tiwari *et al.*[5] demonstrated the antibacterial activity of ZnO nanoparticles against *Acinetobacter baumannii* strain by carrying out the growth disk diffusion assay and growth kinetic analysis. The report of Pavel *et al*.[6] suggested the antibacterial potential of phosphate-based zinc nanoparticles against gram-negative and gram-positive stains. The antibacterial property of ZnO/graphene oxide composites was evaluated against *E. coli* by Yan-Wen and his research group.[7] The antifungal activity of ZnO nanoparticles was inspected against two different pathogenic fungi such as *Penicillium paneum* and *Botrytis cinerea.*[8] Saravana Kumar *et al*.,[9] investigated the antifungal potential of Ni-ZnO nanopartciles against *Candida albicans* fungi. Sanjeevi and his co-researchers[10] reported the efficient antifungal photodynamic potential of cationic Zn-porphyrin against *Saccharomyces cerevisiae*.

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The silver polydiguanides had effectively inhibited the growth of gram-negative strains due to their favourable interaction with the surface of negatively charged bacterial cells.[11] Arivalagan and his co-researchers[12] reported the antimicrobial properties of Fe doped CuO against fungal and bacterial pathogens. The study on the antibacterial potential of Ag coated with glutathione was available in the literature.[13] The bactericidal potential of Ag/transition metal systems was significantly higher against *B. subtilis* and *E. coli* as compared with their individual systems.[14] The nanocomposite of Ag functionalized ciprofloxacin showed a higher antibacterial property against gram-positive and gram-negative strains.[15] The study of Salome *et al.,*[16] demonstrated the enhanced antibacterial activity of Ag-silica nanocomposite compared with conventional agents. The antimicrobial potential of Ag/graphene oxide nanocomposite was analysed against *E. coli* and *S. aureus* by Lei *et al*.[17] Furno and his research team reported that the impregnation of medical devices with Ag enhanced their antimicrobial efficacy and also reduced infection considerably in hospitals.[18] The Ag/PMMA nanofiber was evaluated for antimicrobial activity against Gram-negative and positive bacterial strains.[19]

The organic antibacterial agents have been used in various consumer and health care products like triclosan in hand wash, methylparaben in face cream and diazolidinyl urea in body wash. Generally, microbes develop resistance against these organic antibacterial agents which limit their antibacterial activity. *S. aureus* was one of the microorganisms, which was found to be resistant towards sulfonamides initially. Later penicillin was used to control its growth for which again it developed the resistance. Finally, methicillin was developed in 1960 to stabilize the growth of *S. aureus*, which led to evolution of methicillin resistant *S. aureus* (MRSA).[20] The obtained results of this present research work proved that zinc-incorporated calcium phosphate (Zn-CaB) can be a potential candidate in order to replace the organic antimicrobial agents. The incorporated zinc in the calcium borate is not in the nano range. Therefore, the leaching of zinc does not occur which makes Zn-CaB eco-friendly as well as safer for human beings. In the present work, the prepared Zn-CaB was incorporated into various cosmetic products such as face cream and Body wash to study their thermal stability, photo-stability and antimicrobial performance.

2. Results and Discussion

2.1. Structural and Morphological analysis

The X-ray diffraction patterns of zinc-calcium borate and blank calcium borate are given in **Fig. 1**. The CaB pattern revealed the presence of calcium diborate ($Ca(BO_2)_2$) with JCPDS Card No.:750640 and calcium sodium borate ($CaNaB_5O_9$) phases with JCPDS Card No.780294. On adding zinc into the calcium borates, it was observed that there were no separate reflections due to zinc oxide, suggesting that the zinc has replaced calcium ions in the matrix as reported by earlier workers. This inference was supported by the increase in the intensity of $Ca(BO_2)_2$ in Zn-CaB while the intensity corresponding to $CaNaB_5O_9$ was found to be less. The presence of BO_3 groups in Zn-CaB was concluded by the occurrence of the peaks at 735 cm^{-1} (B-O bending) and 1370 cm^{-1} (B-O stretching)[21] and BO_4 groups were identified by the presence of a peak at 990 cm^{-1} (B-O stretching)[22] (**Fig. 2**). Due to the incorporation of zinc into the matrix of CaB. The asymmetric stretching of B-O was also observed at 1160 cm^{-1}.[23] **Fig. 3** depicts the scanning electron microscope image of Zn-CaB. The morphology of the particle was irregular in shape, and the size of the particle was in the size range of 2 – 3 microns (**Fig. 3**).

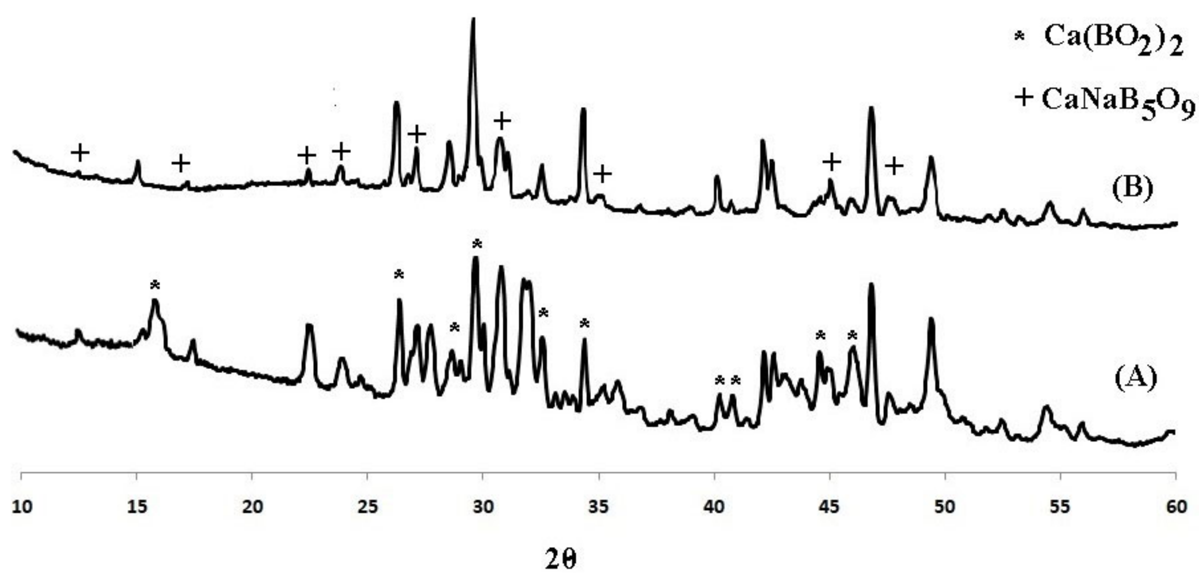

Fig. 1. XRD profiles of calcined samples at 800°C (A) CaB and (B) Zn-CaB

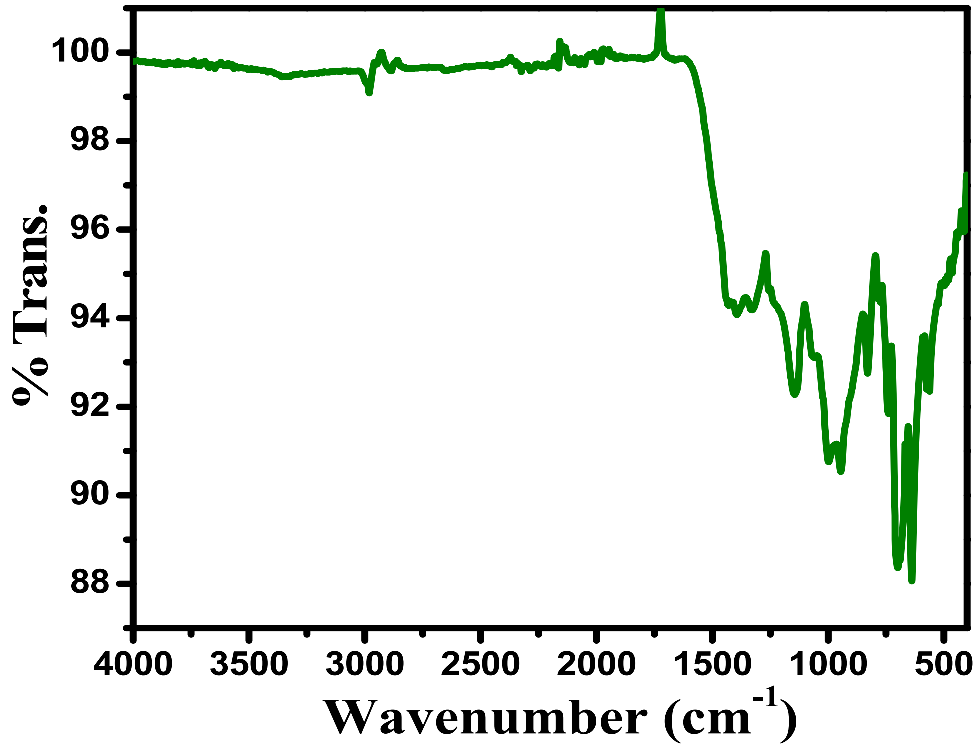

Fig. 2. FTIR spectrum of calcined Zn-CaB at 800°C

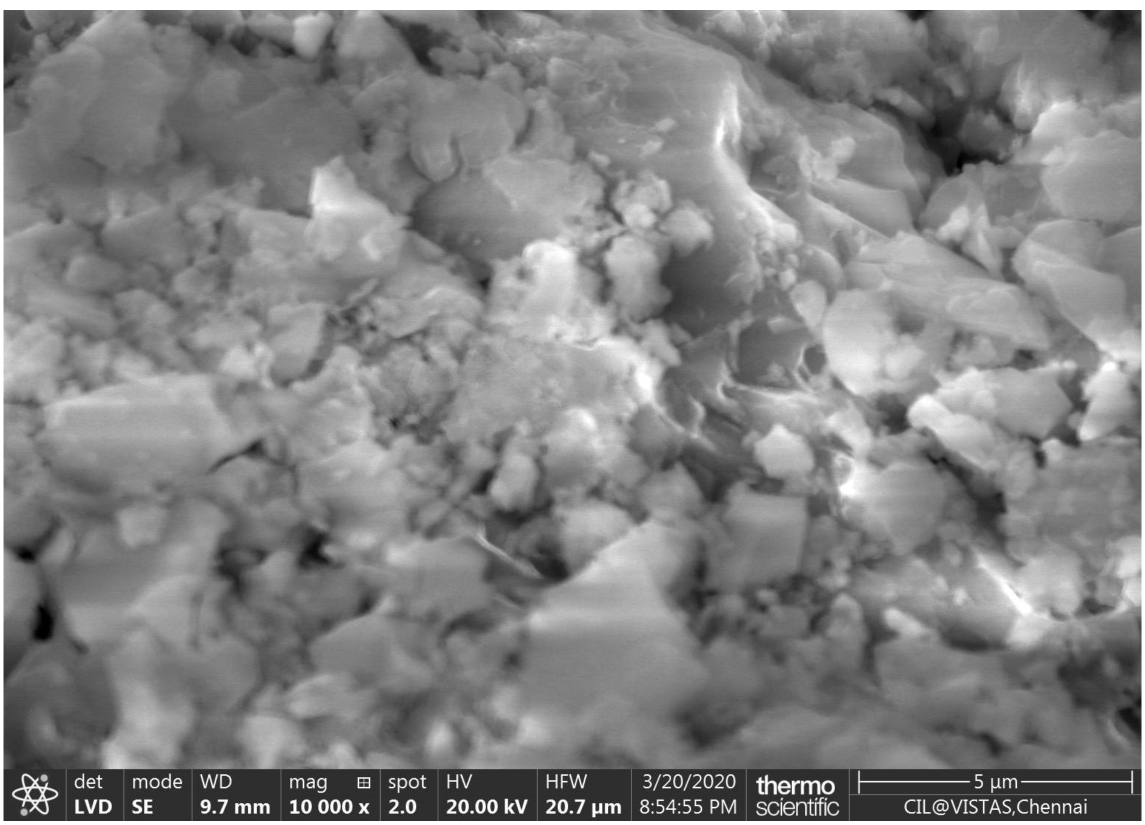

Fig. 3. SEM micrograph of calcined Zn-CaB at 800°C

2.2 UV absorption analysis

Generally, face creams / lotions contain ingredients which absorb ultraviolet rays from the Sun in order to protect our skin from deterioration. Conventionally, various ayurvedic extracts, inorganic chemicals like zinc oxide, and titanium dioxide are used for the above-said purpose. In the present investigation, the Zn-CaB was expected to absorb UV rays, apart from its antibacterial properties. Hence, the UV absorption characteristic of Zn-CaB face cream was measured; its performance was compared with commercially available sunscreen lotion (Himalaya Sunscreen Lotion) and presented in **Fig. 4**. The absorption band of Zn-CaB face cream was broad (200 – 240 nm) as compared with commercial sunscreen lotion (200-220 nm). In addition, the absorption intensity in the present sample was much better than the other sample. It is noted that the UV rays of the Sun lying in the wavelength range of 300 – 400 nm are harmless. However, UV rays of wavelength lesser than 250 nm are most dangerous. The present Zn-CaB is found to be efficient in absorbing a broad range of UV rays of lesser wavelength with increased intensity.

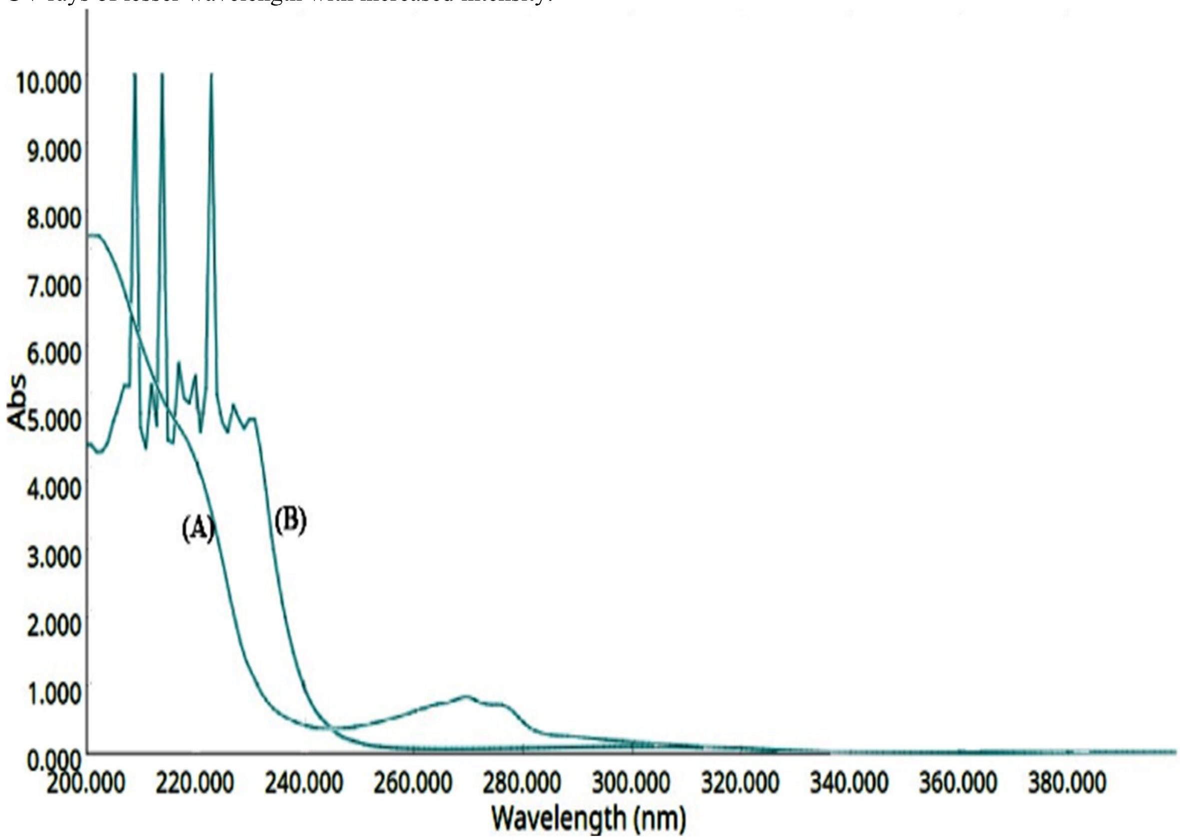

Fig. 4. UV spectra of (A) Commercial sunscreen ointment and (B) face-cream with Zn-CaB

2.3. Antibacterial analysis

The cosmetic products were studied for their antibacterial performance at different concentrations of Zn-CaB, by varying the product content for analysis (**Fig. 5** & **Fig. 6**). The results associated with this analysis are listed in Table 1. Unexpectedly, the pristine cosmetic products (blank) exhibited antibacterial activity, which may be due to the presence of foaming agents and surfactants in the formulations.[24] 1 g of the cosmetic products showed an appreciable activity of around 70%, while the activity of the face-cream was found to be relatively high due to the presence of double the amount of Zn-CaB content than in body wash. The % reduction of CFU/mL was found to increase while increasing the product content for the concentration of given inoculums and the maximum body wash content of 7 g (Zn-CaB=35 ppm) and the face cream content of 3 g (Zn-CaB=30 ppm) exhibited nearly 100% bacterial reduction. For comparison, the commercially available cosmetic products were analyzed which showed nearly 100 % bacterial reduction.

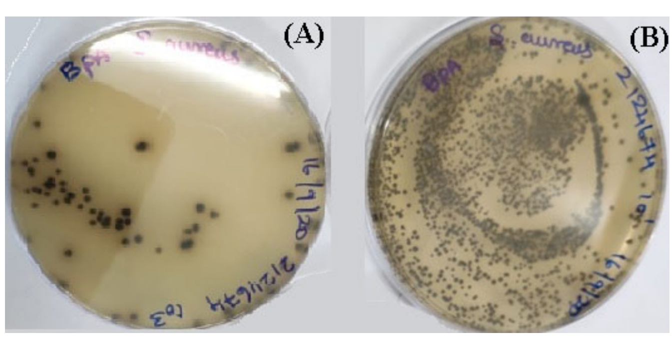

Fig. 5. Antibacterial performance of (A) Body-Wash with Zn-CaB and (B) Blank against *S. auerus*

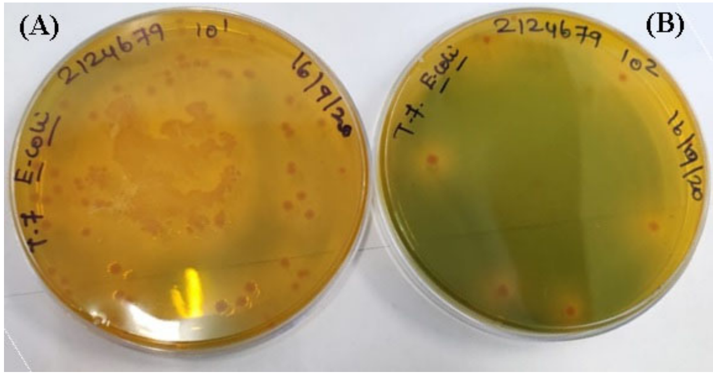

Fig. 6. Antibacterial performance of (A) Blank and (B) Body-wash with Zn-CaB against *E. coli*

Table. 1. Antimicrobial potential of cosmetic products with Zn-CaB

S N	Quantity of Product taken for analysis (g)	BODY WASH *			FACE CREAM *		
		10 mL of Zn-CaB content in culture (ppm)	*S. aureus* (CFU/mL reduction %)	*E. coli* (CFU/mL reduction %)	10 mL of Zn-CaB content in culture (ppm)	*S. aureus* (CFU/mL reduction %)	*E. coli* (CFU/mL reduction %)
1.	1	--	6	11	--	15	18
2.	1	5	62	67	10	72	75
3.	3	15	78	79	30	95	97
4.	5	25	89	89	50	99	99
5.	7	35	99	99	70	99	100
6.	1 (Commercial product)	30 (Active ingredient)	99	98	30 (Active ingredient)	99	99
7.	1 (After stability studies)	5	62	70	10	72	74

* Note: The variation in results was ± 4 units and ± 1 unit for the activity above 97%

2.4. Antifungal analysis

The Zn-CaB incorporated face cream was subjected to antifungal studies (*S. cerevisiae* and *A. brasiliensis*) as illustrated in **Fig. 7** & **Fig. 8.** Table 1 illustrates the results associated with this analysis. An appreciable fungal reduction was observed for 1 g of the sample (Zn-CaB = 10 ppm), which increased on increasing the product content and around 95% of fungal growth was observed for 5 g of the sample (Zn-CaB = 50ppm).

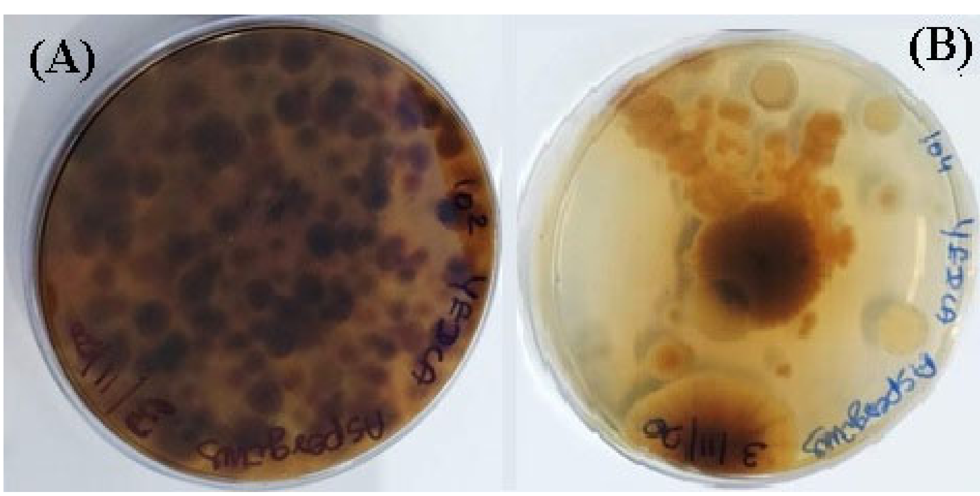

Fig. 7. Antifungal studies against *A. brasiliensis* over (A) Blank (B) Zn-CaB Face cream sample

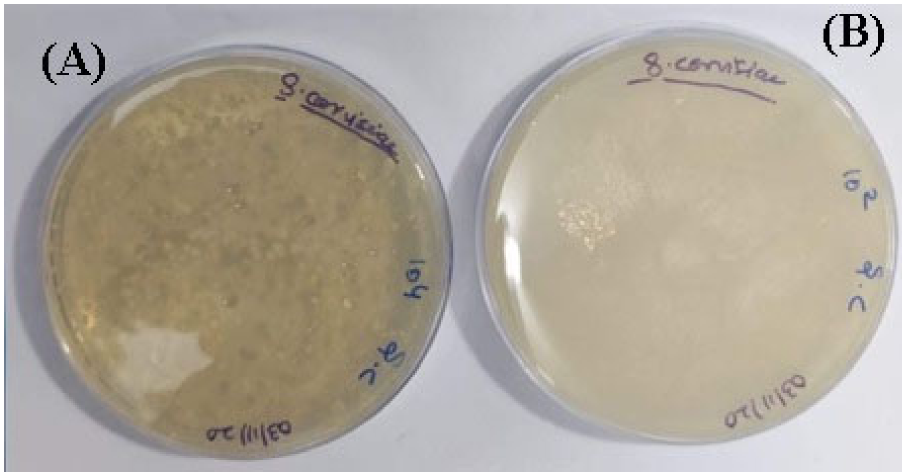

Fig. 8. Antifungal potential of (A) Blank and (B) Face cream with Zn-CaB sample against *S. cerevisiae*

2.5. Stability Studies

Thermal stability and photo-stability of the cosmetic products are important properties required for a commercial product, in addition to appreciable antimicrobial properties. The compatibility of the antimicrobial agent with the matrix of the product is a challenge. In general, organic antimicrobial agents are compatible with the products, since they contain an oil phase, especially in face cream. For inorganic products, it is a general observation that they precipitate out from the matrix. Nanoparticles are compatible due to their smaller size, but they are sensitive towards heat, light and pH. In the present investigation, the thermal stability studies were carried out by subjecting the cosmetic products to different temperatures, viz*.,* 5°C, 30°C and 50°C for 8 weeks. The physical stability, appearance and antibacterial properties of the cosmetic products were examined by testing the withdrawn aliquots of the samples at regular intervals of one week. The results of such a study over the cosmetic products incorporated with Zn-CaB are presented in Table 3. The products were found to be stable without physical separation of phases even after 8 weeks of aging at different temperatures. The physical appearance was also found to be stable and no appreciable colour change was observed.

Table. 2. Antifungal activity of face-cream with Zn-CaB.

Quantity of Product taken for analysis (g)	**Zn-CaB content in 10 mL of the culture (ppm)**	**FACE CREAM***	
		Saccharomyces cerevisiae *(CFU/mL reduction%)*	***Aspergillus brasiliensis*** *(CFU/mL reduction%)*
1	10	67	65
3	30	82	81
5	50	95	94

* Note: The variation in results was ± 3 units

The light sensitivity of the products was studied by exposing them to the UV radiation of 400 nm at 0.75 – 1 m distance for 24 h. The physical stability and colour change of the products were assessed by testing the withdrawn aliquots of the sample for every 8 h. The products made in the present investigation were found to be stable even after 24 of exposure to UV radiation and the antibacterial effect of the samples was retained after subjecting to stability studies (Table 1; Entry-7).

Table. 3. The Stability of the cosmetic products with Zn-CaB

SAMPLE	**Thermal Stability * (After 8 weeks)**		**Photo Stability Studies at RT * (27°C, 24 h)**
	Temperature, °C	**Visual inspection**	
Face Cream Zn-CaB	5	A	A
	30	A	
	50	AB	
Body wash Zn-CaB	5	A	A
	30	A	
	50	AB	

* Code for Discoloration: None – A, Almost Unnoticeable – B, Slight – C, Noticeable – D, Distinct – E and Severe - F

Despite having PPE, there are good chances for the microbes to be attracted to the surface of the clothes and progressively infect the person. Hence, the purpose of adding Zn-CaB in detergent is to incorporate a relatively prolonged antibacterial effect on the fabric. Generally, the detergents contain harsh chemicals like soda ash, linear alkylbenzene sulfonic acid.[25] But, it exhibits a bactericidal effect and does not give a bacteriostatic effect. Table 4 compares the bacteriostatic effect of blank detergent and detergent incorporated with Zn-CaB. Around 60 cm x 60 cm of rayon fabric was washed with around 5 g of blank detergent in an automatic washing machine with the normal running speed. Another rayon fabric of the same dimension was washed with around 5 g of Zn-CaB detergent under the same conditions. The fabric was dried in shade under a dust-free atmosphere. A small piece of fabric of around 10 cm x 10 cm dimension was removed from both the fabric using sterilized scissors and tested for the presence of microbes. The procedure involves taking around 30 mL of sterilized saline water in a sterilized conical flask. The sample fabric was introduced into the conical flask and plugged with sterilized non-absorbing cotton and shaken well for around 15 minutes. This process leaches the microbes present on the fabric into the saline water. 1 mL of the saline water from the conical flask was introduced onto the petri plate containing nutrient agar and incubated for 48 h. The microbes, if any, present in the saline water grow over the nutrient agar, and the CFU/mL is noted and reported.

Table. 4. Antibacterial analysis of fabric washed with Zn-CaB detergent.

SN	No of Washing Cycles tested after 15 days of exposure to air in shade	Blank detergent	Zn-CaB detergent
1.	0	0	0
2.	1	5	0
3.	2	27	0
4.	3	55	4

Initially, the fabric washed with blank detergent and Zn-CaB detergent showed no growth of microbes. Both the fabrics were exposed in the atmosphere under shade for 15 days and again determined for microbial growth. No microbial growth was observed for both the fabric (Table 4; Entry-1). The fabrics were washed again separately in the washing machine without adding detergent, and the procedure was repeated. The results are reported as Cycle 1 (Entry-2), where blank detergent showed the development of few colonies and the Zn-CaB detergent exhibited bacteriostatic effect. Again the fabrics were washed separately in the washing machine, without adding detergents and microbial growth was determined. A substantial increase in the CFU/mL was observed for the fabric washed with blank detergent while the bacteriostatic effect was observed up to 4 washing cycles for the fabric washed with Zn-CaB. Thus, the antimicrobial effect was incorporated in the fabric for a relatively long period as compared to blank detergent.

3. Conclusions

Zinc incorporated calcium borates were successfully prepared by co-precipitation technique, where zinc ions were found to replace calcium ions in the calcium borate matrix as confirmed by XRD and FTIR vibrational analysis. The particle size of the Zn-CaB was found to be in the range of 2 - 3 microns by SEM. The UV rays absorption property of Zn-CaB face cream was equivalent to commercially available sunscreen lotion. The body wash and face-cream showed >99% reductions in bacterial growth with 30 ppm and >95% reduction in fungal growth with 50 ppm of Zn-CaB in the products. The Zn-CaB incorporated personal care products in the present investigation were thermally stable and no photo-degradation was observed. The products were found to retain its antimicrobial activity after ageing for more than 8 weeks at elevated temperature. The Zn-CaB blended detergent incorporated a bacteriostatic effect on the fabric for more than 3 washing cycles. Thus, Zn-CaB acts as a promising antibacterial agent in the personal care products, and further clinical studies are under progress.

4. Experimental

4.1 Materials

Zinc nitrate hexahydrate (99%), sodium tetraborate and sodium carbonate were received from Nice chemicals, India. The other chemicals like Cetyltrimethylammonium bromide, calcium nitrate tetrahydrate (98%), EDTA disodium salt (99%), potassium hydroxide (99.9%), glycerol (87%), and sodium chloride (99.5%) were obtained from Merck, India. The chemicals required for the preparation of cosmetic products like sodium laureth sulphate, cocamidopropyl betaine, stearic acid, isopropyl myristate, glycerol monosterate, soda ash, sodium sulphate, linear alkylbenzene sulfonic acid, sodium tripolyphosphate, triple superphosphate were purchased from bulk manufacturers through commercial sources.

4.2 Preparation of zinc doped calcium borates (Zn-CaB)

The incorporation of zinc into calcium borates was achieved by dissolving zinc nitrate hexahydrate (7.83g) and calcium nitrate tetrahydrate (23.6g) in 100 mL of distilled water. In a 500 ml beaker, 19.05 g of sodium tetraborate decahydrate was dissolved in 200 mL of distilled water at 80°C under constant stirring. To the above solution, 0.1 g of cetyltrimethylammonium bromide (99%) was added in the stirring condition. The zinc and calcium nitrate solution was slowly added to the borate solution under constant agitation to form a white precipitate. After the complete addition of nitrate solution, the stirring was continued for one hour and the obtained precipitate was left undisturbed for 12 h. Then the collected supernatant was examined by the addition 0.1 N solution of sodium carbonate to conclude the presence of any calcium and/or zinc ions in it. The precipitate was centrifuged and washed with distilled water repeatedly to remove residual nitrate ions. Then it was dried at 120°C for 6h. Further, it was calcined for 30 min at 800°C to obtain the zinc doped calcium borates (Zn-CaB).

4.3. Characterization

The presence of calcium borate phases was identified by recording XRD patterns on a Brucker K 8600 instrument. The bonding in the calcium borates incorporated with zinc was confirmed using FTIR spectroscopic analysis (Shimadzu 8400S, Japan). The morphology was studied by capturing an SEM image using JSM 6300, JEOL, USA. The UV absorption behaviour of the Zn-CaB incorporated face-cream was studied using Systronics Type 2202 double beam UV-Visible spectrometer.

4.4. Preparation of Cosmetic products

The Body wash was prepared by dissolving 18g of sodium laureth sulphate (surfactant and cleaning agent) in 36 g of distilled water. To the above 5 g of cocamidopropyl betaine (foaming agent) was added and stirred gently to avoid the formation of foam. After complete mixing, around 1.5 g of glycerol was added as a humectant, whence, the solution gets warm. After cooling, around 1 g of sodium chloride was added to enhance the viscosity of the gel and the contents are made up to 56 g with distilled water. Finally, 0.5 grams of Zn-CaB (antimicrobial agent) was added as a fine powder and blended gently for 1 hour.

The face cream was prepared by dissolving 0.25 g of EDTA (in order to soften the water) in around 70 g of distilled water at around 75°C. In another container, 16 g of stearic acid (emulsifying agent) was heated and liquefied at around 80°C. To this, 1.25 g of isopropyl myristate (to improve thixotropic behaviour), 1 g of glycerol monosterate (self-emulsifier) was added and blended under hot condition to form a thick oil phase. In the third container around 0.5 g of potassium hydroxide (neutralizer) was dissolved in around 10 g of water and kept separately. Under the hot condition, the oil phase was added to the EDTA solution under constant agitation for around 60 min to form a homogeneous gel. The potassium hydroxide solution was added in drops and agitated constantly to get pH between 6.5 and 7.0. Finally, 1 g of Zn-CaB was added and blended till homogenization to form the final face cream. The Zn-CaB content was increased in the face cream due to the dual role of antibacterial as well as UV protection, while the commercial face creams generally contain nearly 3% of the active ingredient.

The detergent for the present investigation was made by blending 70 g of commercial-grade soda (cleaning agent) with 2 g of sodium sulphite (oxygen scavenger to prevent corrosion). Around 20 g of linear alkylbenzene sulfonic acid (mercerizing agent) was added slowly under constant blending. To the above content, around 3 g of sodium tripolyphosphate and 2 g of triple superphosphate (chelating agent) were added to enhance penetration as well as to provide whiteness for the fabric. Finally, it was added with 3 g of Zn-CaB and blended for 30 min.

Fig. 9A. Face-cream with 1% of Zn-CaB

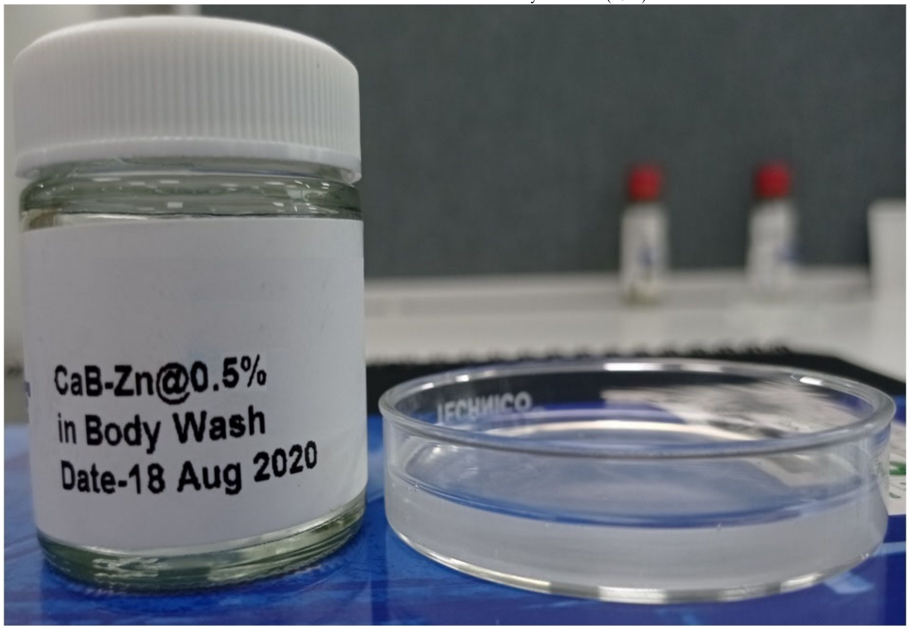

Fig. 9B. Body wash with 0.5% of Zn-CaB

4.5. Antibacterial study

The antimicrobial potential of the cosmetic products was evaluated by ASTM-E2149 standard. The pathogenic strains used for the analysis were *Escherichia coli* (NCIM 2931), and *Staphylococcus aureus* (NCIM 2127). The initial concentration of pathogenic strains was maintained as 115 X 10^5 CFU/mL for *E. coli* and 57.5 X 10^5 CFU/mL for *S. aureus.* The typical procedure involves the transferring of inoculums (1 mL) into a sterilized conical flask containing 9 mL of distilled water (sterilized). The cosmetic products (1 g) were transferred into the conical flask plugged with sterilized non-absorbing cotton and shaken for 60 min in a wrist-shaker. After 1 h, 1 mL of the product was added to the nutrient agar plate by using an L rod. The plate was covered with another plate and incubated in the inverted position at 37°C for 24 h. The antibacterial activity was reported as percentage colony forming units per mL (CFU/mL) in comparison with blank. The experiment was duplicated. The antifungal activity of the cosmetic products was analysed against *Aspergillus brasiliensis* (NCIM 1196) fungal concentration of 8.7 X 10^4 CFU/mL and *Saccharomyces cerevisiae* (NCIM 3287) fungal concentration of 5.32 X 10^5 CFU/mL by incubating them for 5 days.

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