

CHAPTER-8

FORMULATION DESIGN DEVELOPMENT



8 Formulation Design, Development & Evaluation

Naturopathic physicians contend that most diseases are the direct result of the ignorance and violation of what would be traditionally called “natural living laws” and so ideally, naturopathy involves only the use of therapies that support the human beings and encourage nature’s intrinsic healing process to work more effectively. Therefore the medicines prescribed by them are primarily natural and relatively unprocessed. Processing the natural element (plant, animal, or mineral source) by isolating the active ingredient from it, means breaking the natural configuration which nature has made for the living system to survive. Thus, use of such type of isolated natural elements for human use means disrupting the natural body mechanism. Mud is one such natural element that has been traditionally used for treatment of various skin disorders, in form of mud baths. Hence, our aim was to design formulation of mud without drastically altering its natural properties.

Also there are certain limitations of full body mud bath (mud therapy).

(1) One has to preplan In advance to have mud therapy. Also mud bath is very time consuming. Moreover, mud may choke the drainage pipelines if therapy is taken at home. Therefore It has to be taken in a Nature Cure Center where special arrangement is made to drain applied mud. Further, it becomes difficult for individual person to get huge stock of mud needed for mud bath.

(2)The actual time for the treatment includes, time for wetting mud (15 min), time for application (approx. 20 min), time for drying (approx. 30 min), time for removal (approx. 30 min bathing time) i.e. total of approx.1hr 35 min. and so it is difficult to spare so much time , daily, in this busy life.

(3)When mud is applied on body, it is in thick layers, which may fall off the body when a patient moves about. As a result, the patient’s **mobility is restricted**. Thus it can be seen that mud bath therapy is not a patient friendly treatment. Hence we

tried to prepare patient friendly dosage forms, like paste, lotion, patch, powder and spray, from cheapest, easily available, non toxic, and self prescribable medicine 'mud' which otherwise has seen limited use because of its patient's inconvenience in application. These formulations were also expected to overcome the limitations of mudbath therapy, save time, not affect the patient's mobility and also be able to provide uniform application of mud to all affected body parts.

From literature review, we found that different formulations of mud are available in the foreign market but in India only paste is manufactured ('MUD' by Himalaya Drug Co.). Formulations like thermal mud soap, face pack, moisturizer night cream, mud pack with moisturizer, face scrub and face pack, mud face pack with aloe vera and vitamin E etc are manufactured in New Zealand and other European countries. Several preparations made from mud are also patented (US patent 6582709, US patent 5705172, US Patent 20070212434, & US Patent 6267962). Hence we aimed to design and develop mud –based formulations like **Pastes, Lotions, mud patch, spray and dry powder** with the aim to overcome the limitations of restricting mobility, to be better accepted by patients as compared to conventional method of application of mud and to overcome the variability in application.

Development and Evaluation of formulations

Formulations like pastes, lotions, powders, mud patch and spray were designed and developed.

8.1 Paste

Pastes of all four muds were prepared by adding sufficient vehicle to mud to get paste like consistency. As each mud had different water holding capacity, each batch was standardized with respect to water for achieving the desired consistency of the paste.

Formula:

Mud : passed through 120#

Methyl paraben : 0.1% w/w

Propyl paraben : 0.1% w/w

Glycerin: 8-10% w/w of mud

Distilled water; q.s. For achieving paste like consistency

Parabens were first dissolved in small quantity of water and added to mud. Glycerin was mixed into it and water was then added slowly with constant stirring to prevent formation of agglomerates.

8.1.1 Optimization

Concentration of methyl paraben, propyl paraben, water and glycerin were optimized.

8.1.1.a Preservatives

Though mud itself has antimicrobial activity, preliminary studies had shown presence of spores present in the soil which would germinate in moist environment of pastes. So addition of preservatives was found necessary.

Different concentrations of methyl paraben and propyl paraben were investigated. (0.05%, 0.1%, 0.2%, 0.25%) each of methyl and propyl parabens were added to the paste. This paste was stored in sterilized air tight plastic container for six months. It was opened in a sterile environment every 30 days to observe any microbial or fungal growth for six months.

Observation

It was observed that there was fungal growth in all the four muds having 0.05% each of methyl and propyl parabens while there was no fungal growth nor any visual degradation (change in appearance or consistency of paste or foul smell due to

bacterial degradation) at higher levels , 0.1% 0.2% and 0.25% each of parabens. Thus, 0.1% each of methyl and propyl paraben was considered to be optimum preservative concentration.

8.1.1.b Glycerin

Glycerin was added to the formulation to act as a humectant to prevent the paste from drying in the container as well as on the skin. Different percentages of glycerin i.e. 5%, 10%, 15%, 20%, v/w of mud was added to all four muds and drying time was observed.

Each mud was applied on dorsal part of hand (3 subjects) in 1 inch square area of a thickness of 1mm. Drying time (i.e. drying until formation of cracks) was noted and shown in table 47.

Table no.47 Effect of glycerin on drying time of pastes

Sr.no.	Paste	Drying time (min)			
		5%	10%	15%	20%
01	Kerala Black	15±2	30±4	65±4	90±6
02	Kerala Brown	17±3	32±2	70±5	90±7
03	Dwarka	15±1	30±2	60±4	90±5
04	Vadodara	13±4	25±5	50±6	80±4

It was observed that drying time increased as concentration of glycerin increased. Also, drying time was comparatively less in Vadodara paste. Since clay content was least in Vadodara, its drying time was shorter than the others. As 30 minutes time was required for mud therapy using formulations, 10% of glycerin was considered optimum concentration for preparing pastes.

8.1.1.c Water

The quantity of water added to the paste was judged on the basis of the final consistency of the paste. This was monitored by rheological analysis. The rheological property of paste was measured after one day of preparation, to allow maximum swelling of mud. The study was conducted at Geotechnical Dept. of Faculty of technology & Engineering, M.S.University of Baroda using Brookfield viscometer RV model, spindle no.7 using various rpm. The viscosity of each mud at various rpm is as shown in table no.48 and fig. no.63.

Table no 48 Rheology data of four pastes (viscosity in centipoises)

RPM	Black	Brown	Dwarka	Vadodara
0.5	136000	200000	120000	240000
1	320000	420000	300000	520000
2.5	304000	376000	312000	352000
5	256000	300000	272000	256000
10	200000	154000	216000	136000
20	90000	88000	112000	76000
50	44000	44800	52000	30800
100	32000	31800	38000	27200

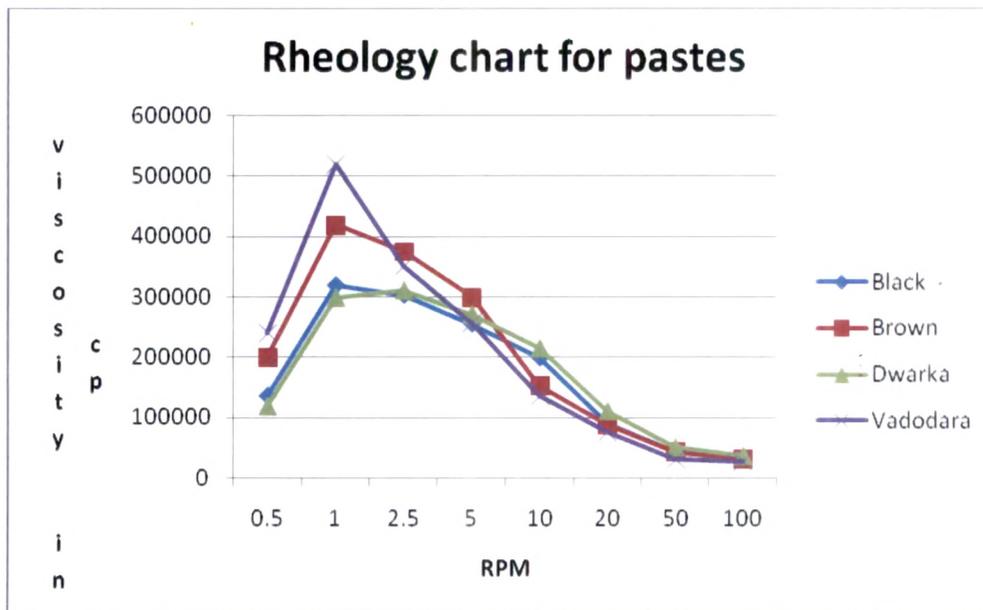
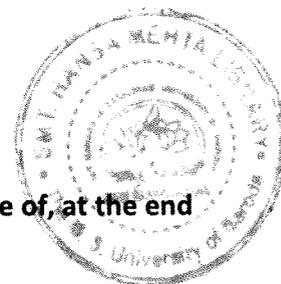


Fig.no 63 Rheology chart of pastes

It was obvious from the fig. 63 that all the pastes showed pseudoplastic flow (shear thinning). Each paste had a sharp rise in viscosity initially but after that point the viscosity decreased as shear rate increased. This implied that initially, more stress will have to be applied to extrude paste from collapsible tubes. Once the initial flow has commenced, further extrusion becomes easy and at the same time, when the force applied is withdrawn, the paste too will stop flowing and withdraw into the tube thus preventing wastage of it when it is not required.

Viscosity at 1 rpm was considered to characterize the flow. The water needed to produce viscosity of 320000, 420000, 300000, 520000 cp at 1 rpm for Black, Brown, Dwarka, Vadodara pastes respectively, was considered as optimum water to be added to make paste of required consistency. Thus optimum water content to be added was found to be in the range of 100-150ml for 400 gm of mud.



8.1.2 Stability

The samples were examined monthly and the reports given here, are of, at the end of the study.

8.1.2.a Color, Odor, Visual appearance

There was no change of colour or visual appearance nor was there was any foul odor in the pastes after six months. This indicated that there was no chemical or microbial degradation in the pastes, showing their physicochemical stability at room temperature.

8.1.2. b. Loss of water

The prepared pastes were filled and sealed in aluminium collapsible tubes. They were weighed initially and after six months of storage at room temperature. The % loss of water was calculated and tabulated in Table no.49.

Table No: 49 % loss of water on storage

Sr.no.	Pastes	% loss of water
01	Kerala Black	0.158±0.01
02	Kerala Brown	0.692±0.002
03	Dwarka	0.035±0.009
04	Vadodara	1.23±0.02

Result

It was observed that there was relatively very less water loss from all the pastes (0.035 to 1.23%), indicating their good water retention capacity. Vadodara paste

showed maximum loss, probably because of the least clay content, which plays an important role in water retention.

8.1.2.c Microbial Examination(IS 14648:2005)

The pastes used for the testing were stored in collapsible aluminium tubes for six months. The tubes were opened in aseptic environment to draw out pastes for monthly testing. 5 gms of each paste was taken in a sterile container and shaken with 10 ml of Sterile distilled water and 2 ml of Tween 80 (to neutralize the effect of methyl & propyl parabens) on a test tube shaker(vortex shaker) for 5 minutes. This suspension was stored in an aseptic cabinet for 120 mins. for contact time. One loopful of the supernatant of the pastes' suspension was inoculated on Blood Agar, MacConkey's Agar and Nutrient Agar plates in triplicate. These plates were incubated at 28^o & 37^o C for 48hrs and then observed for microbial growth.

Positive controls in triplicate using *S. aureus* were also incubated for 48 hrs. at 28^oC and 37^oC.

Result:

No growth was found in the plates upto six months, indicating that the concentrations of methyl and propyl paraben were adequate to preserve the paste formulations.

8.1.2.d. pH

The pastes were diluted with water (1:2 paste : water), and the mixture was shaken on vortex shaker to ensure complete mixing. The supernatant was used to measure pH by Elico pH meter. The results are shown in table no.50.

Table no. 50 pH of pastes (stability studies)

pH	Kerala Black	Kerala Brown	Dwarka	Vadodara
Initial	5.89±0.02	7.39±0.01	7.34±0.01	7.79±0.03
After 6 months	5.91±0.03	7.38±0.04	7.39±0.03	7.62±0.01

There was no significant change in pH of the pastes after six months of storage, further proving the chemical stability of the mud in the pastes.

8.1.2.e. Texture

Topical semisolids should be soft, smooth and non-gritty in nature. On storage after six months, the pastes were felt between the fingers and found to be non-gritty, soft and smooth.

8.1.2.f Spreadability

About 1 gm of each sample was weighed and placed at the centre of the glass plate (10x10 cm) and another glass plate was placed over it carefully. Above the glass plates 2 Kg. weight was placed at the centre of the plate. Sliding of the plate was avoided. The diameter of the paste in centimeters was measured after 30 min, for all samples (Akelesh T 2010). The experiment was performed in triplicate and the results are shown in table no. 51.

Table no. 51 Spreadability of Pastes

Mud	Spreadability in cms.
Kerala Black	4.2±0.2
Kerala Brown	4.9±0.1
Dwarka	4.5±0.2
Vadodara	3.9±0.3

It was observed that all the pastes had good spreadability (Akelesh T 2010). As specified in the Bureau of Indian standards IS 6356-1993, the maximum spreadability allowed is 8.5 cms. This indicated that our pastes passed this test.

8.2 Lotions

Lotions would be preferred by those patients who have large disease patches and those who do not have time to spare for full body mud bath. Lotions are fluid and can form a thin layer on the body when dried. Also, this can be removed by just rubbing off with hand or tissue and there is no need to wash or rinse with water. Hence, lotions can be used even during day time and can be more user convenient.

Preparation

Soil : 200gm (Less than 75um particle size i.e. passed through 200# sieve)

HPMC (Hydroxy propyl methyl cellulose , Make: Loba Chemie , E 50LV Premium) :
2% weight of soil

Methyl paraben ; 0.1% w/w

Propyl paraben : 0.1%w/w

Distilled water : quantity sufficient

Methyl and propyl parabens, and HPMC were dissolved in small quantity of water and added to soil. The remaining water was then added slowly (to attain lotion like consistency), with constant stirring to prevent formation of agglomerates and filled in plastic air tight container. Instruction "Shake well before use" was labeled on it.

8.2.1 Optimization of water

Rheology characteristics of lotion was studied (Brookfield RV model, spindle no. 7) in the same manner as pastes and optimum water concentration was found out. All the lotion showed pseudoplastic flow and viscosity at 1 rpm was used to characterize the flow. The viscosity of Kerala Black, Brown, Dwarka and Vadodara was found to be 2,20,000, 2,40,000, 2,10,000 and 2,30,000 cp respectively.

8.2.1.a. Stability

All the stability tests were conducted every month and the reports given here are those of , at the end of six months.

8.2.1.b Caking

Caking property of the lotion is decided by its Zeta potential value.

Zeta potential of soils were measured by Malvern zeta sizer (Make: Malvern Master Sizer) using distilled water as dispersant. The reports of Zeta Potential of Black, Brown, Dwarka, and Vadodara are given in fig.no.64, 65, 66, and 67 respectively and in table no.52.

Table no: 52 a Zeta potential of lotions

Mud	Kerala Black	Kerala Brown	Dwarka	Vadodara
Zeta potential mV	-14.1	-26.6	-16.4	-22.7

It could be observed that Kerala Brown had highest zeta potential followed by Vadodara which indicated least caking quality which was confirmed by redispersibility studies.

8.2.1.c Redispersibility studies

After storage for about 18 hours at room temp., the containers (100ml, lotion vol.50ml) were repeatedly inverted and the number of inversions required for redispersing the solid mud was counted. The results are shown in table no.52 b. It could be observed that Kerala Brown and Vadodara lotions were completely redispersed by 4 inversions and Kerala Black needed 6 shakings. This is in co-ordinance with the zeta potential readings.

Table no: 52 b Redispersibility test

Mud	Kerala Black	Kerala Brown	Dwarka	Vadodara
No. of inversions	6	4	5	4

8.2.1.d Sedimentation Volume

Sedimentation volume was measured after every 4 hrs. For the first 4 hrs. the vol. was 0.98, 0.96 after 12hrs. and 0.9 after 24 hrs which remained constant after 36 hrs. for Dwarka and nearly same pattern was observed for other three lotions. The final sedimentation volume after 36 hrs. for all the other lotions is shown in table no.53. Thus it could be observed that there was very little sedimentation on storage. Negligible sedimentation may be attributed due to high suspending properties due to presence of polymer like humic acid which is a natural suspending agent and addition of HPMC, which acts as viscosity enhancer.

The high negative Zeta potential values may also have contributed to the physical stability of the suspensions by charge repulsion stabilization mechanism.

Table no.53 Sedimentation volume (lotion)

Mud	Kerala Black	Kerala Brown	Dwarka	Vadodara
Sedimentation volume After 36hrs	0.94±0.01	0.95±0.03	0.9±0.03	0.92 ±0.02

Zeta Potential Report

v2.2



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Sample Details

Sample Name: Kerala Black 1
 SOP Name: miansettings.dat
 General Notes:

File Name: Beena.dts Dispersant Name: Water
 Record Number: 6 Dispersant RI: 1.330
 Date and Time: Wednesday, January 19, 2011 7:00:00 AM Viscosity (cP): 0.8872
 Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0 Zeta Runs: 12
 Count Rate (kcps): 274.7 Measurement Position (mm): 2.00
 Cell Description: Clear disposable zeta cell Attenuator: 7

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -14.1	Peak 1: -14.1	100.0	3.91
Zeta Deviation (mV): 3.91	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.0481	Peak 3: 0.00	0.0	0.00
Result quality : Good			

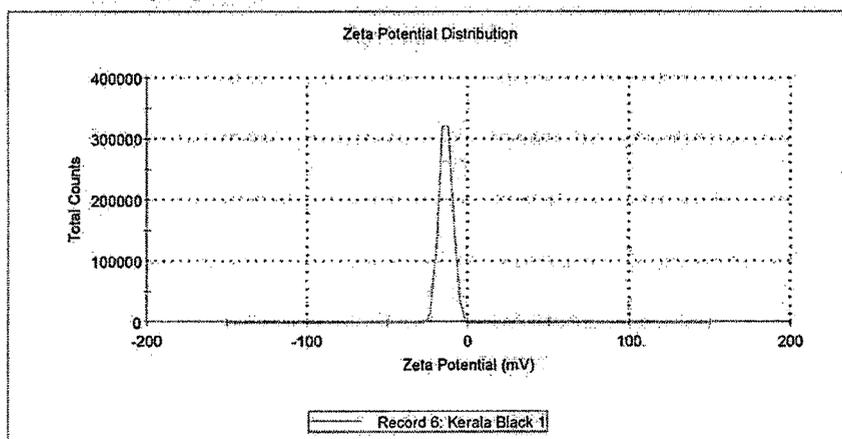


Fig.no 64 Zeta potential report of Kerala Black

Zeta Potential Report

v2.2



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Sample Details

Sample Name: Dwaraka 1
SOP Name: mansettings.dat
General Notes:

File Name: Beena.dts Dispersant Name: Water
Record Number: 3 Dispersant RI: 1.330
Date and Time: Wednesday, January 19, 2011 6:... Viscosity (cP): 0.8672
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0 Zeta Runs: 12
Count Rate (kcps): 180.4 Measurement Position (mm): 2.00
Cell Description: Clear disposable zeta cell Attenuator: 9

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -16.4	Peak 1: -16.4	100.0	4.75
Zeta Deviation (mV): 4.75	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.0186	Peak 3: 0.00	0.0	0.00
Result quality: Good			

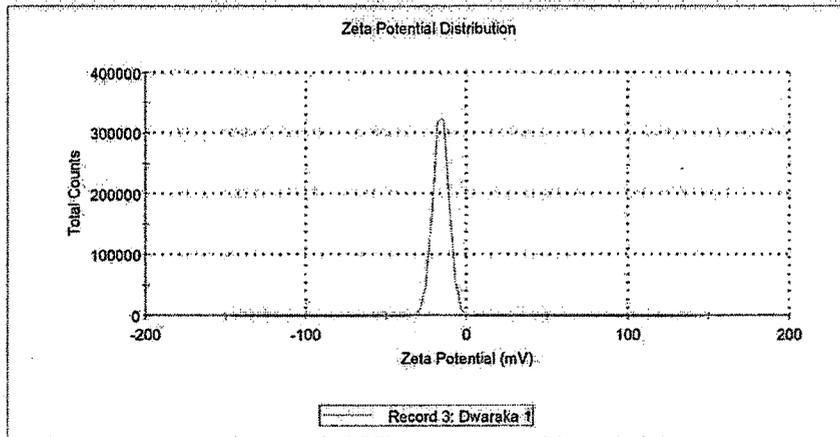


Fig.no. 66 Zeta potential report of Dwaraka

Zeta Potential Report

v2.2



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Sample Details

Sample Name: Vadodara-1
 SOP Name: mansettings.dat
 General Notes:

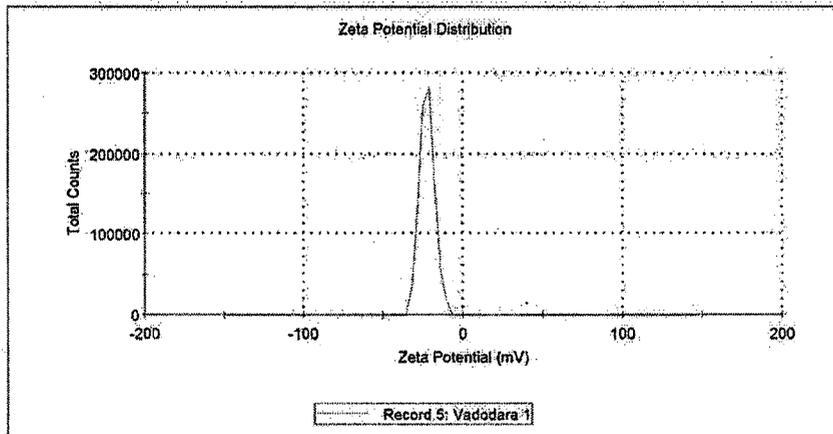
File Name: Beera.dts Dispersant Name: Water
 Record Number: 5 Dispersant RI: 1.330
 Date and Time: Wednesday, January 19, 2011 6:... Viscosity (cP): 0.8872
 Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0 Zeta Runs: 12
 Count Rate (kcps): 190.9 Measurement Position (mm): 2.00
 Cell Description: Clear disposable zeta cell Attenuator: 6

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -22.7	Peak 1: -22.7	100.0	4.70
Zeta Deviation (mV): 4.70	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.0107	Peak 3: 0.00	0.0	0.00
Result quality : Good			



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DPS Ver. 5.10 beta.1
 Serial Number : MAL501131

File name: Beera
 Record Number: 5
 24 Jan 2011 6:51:20 PM

Fig.no. 67 Zeta potential report of Vadodara

Stability studies:

Similar to pastes, the lotions were also subjected to stability testing up to 6 months and evaluated for physical observation, microbial testing and pH.

8.2.2.a Physical observation

There was no change in color, odor, and physical appearance of the lotions upto 6 months storage at room temp. except settling, which was redispersed.

8.2.2.b Microbial Testing

After six months of storage, 5 ml of each lotion was taken in a sterile container and shaken with 10 ml of Sterile distilled water. 2 ml of Tween 80 (to neutralize the effect of methyl & propyl parabens) was added to it and shaken on a test tube shaker (vortex shaker) for 5 minutes. This suspension was stored in an aseptic area for 120 mins .for contact time. One loopful of this supernatant was inoculated on Blood Agar, MacConkey's Agar and Nutrient Agar plates in triplicate. These plates were incubated at 28^o & 37^o C for 48hrs and evaluated for microbial growth.

Positive controls in triplicate using *S. aureus* were also incubated for 48 hrs. at 28^oC and 37^oC.

Observation and results:

No microbial growth was observed in any of the samples, indicating efficacy of the preservatives added.

8.2.2.c pH

1:2 dilution of lotions was made and pH of the supernatant was determined pH by Elico pH meter. From the results shown in table no.53 it was observed that there was no significant change in the pH after storage, indicating no degradation during storage period.

Table no: 54 pH of Lotions (stability studies).

pH	Kerala Black	Kerala Brown	Dwarka	Vadodara
Initial	5.89±0.02	7.39±0.01	7.34±0.01	7.79±0.03
After 6 months	5.93 ±0.03	7.41±0.04	7.39±0.03	7.72±0.01

8.3 Mud compresses

It is well established that occlusion of skin improves penetration of molecules as compared to that of non-occlusive formulations. Occlusion leads to hydration, prevents drying of skin and opens up the pores, thus increasing the permeability of the skin. At the same time, use of an adhesive patch helps the dosage form stay at the affected site for a very long period and is not affected by the environment, washing, physical activity etc. All this will improve patient convenience and compliance and also enhance therapeutic outcome. Hence, we designed Mud packs or compresses similar to adhesive patches.

8.3.a Preparation :

Two pieces of white thin cotton cloth(fig. no 69) was cut into 5cm x 4cm pieces.10 gm of paste (Prepared by the method described earlier) was spread on one of it , leaving 1 cm border on all four sides . The other piece was put on it to cover the paste after 10 min. so that cloth absorbs the water of the paste. This piece was then stuck on Surgipore adhesive tape (6 cm breadth).(fig.no.68). HDPE(High density polyethylene) plastic was taken (3 cm longer than the size of the tape) and cut into two halves . The two halves were stuck on the patch in such a manner that there was overlapping of 1cm in the middle of the patch (fig.no.70).The purpose of overlapping of plastic was to assure complete covering of the paste (protection from

external environment) and easy lifting of the plastic to hold, to apply on the skin (fig. no. 71). At the time of application, after opening the flaps, little water was sprinkled just enough to moist the paste and then the patch was ready to use (fig.no.72).



Fig. no. 68 Surgipore adhesive tape



Fig.no.69 Checked cotton cloth

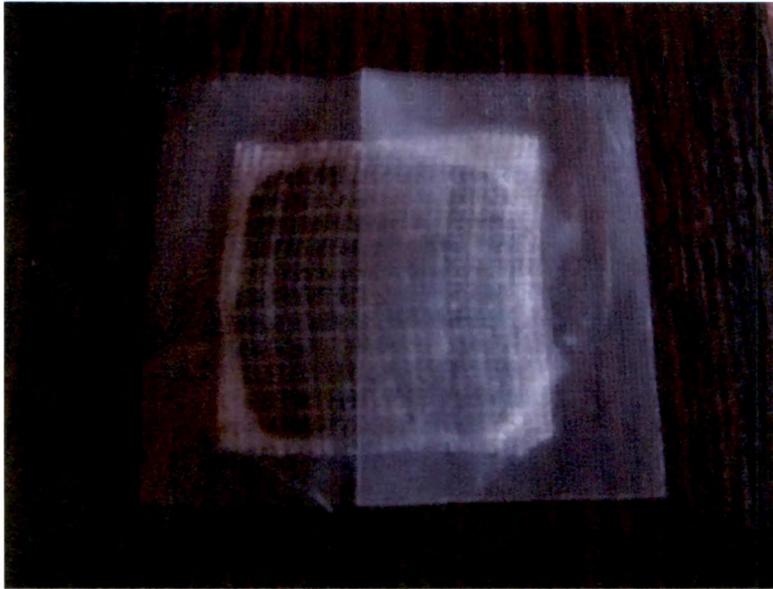


Fig. no.70 Mud compress



Fig.no.71 Opening of the two flaps



Fig.no.72 Application of mud compress

Different sizes and shapes of mud pack were prepared. Patches were stored in flat air tight containers to prevent loss of moisture.

8.3.b. Stability:

The patches were examined after every month to verify intactness or cracking or drying etc of paste cake in the compress. The adhesion property of the adhesive tape was also checked for each patch tested. After six months of storage, there was no change in the cake of mudpaste and the adhesiveness of tape was also not reduced.

8.4 Powder

Powder of mud (passed through 500#, <25um size) was used as such without adding any extra ingredient. Powder was filled in a air tight container with sprinkler lid or in a shallow, wide mouthed container with powder puff for application. According to the container, the patient was instructed to either sprinkle it over the affected body part or apply by a puff on the affected part. Since it was a very fine powder, it was cosmetically accepted by patients with lesions on face.

8.4.1 Evaluation properties:

8.4.1.a Flow characteristic

Angle of repose was found out for the powders by funnel method. Glass funnel was fixed at height of 3 cm from bottom and powder was allowed to flow freely. The height and radius of the pile was measured and angle of repose θ is calculated by $\tan\theta = \text{height}/\text{radius}$. The results are shown in table no. 54. Since the particle size of the powder was very small, the flow property was poor (excellent flow is between 20-25).

Table no. 54 Angle of repose of soil powders

Mud	Kerala Black	Kerala Brown	Dwarka	Vadodara
Angle of repose	16	18	14	19

8.5 Spray

Sprays are popular fluid dosage forms which have multiple advantages: they can be applied in very thin uniform layers, they can cover large body surface area, they are hygienic as they can be applied without using hands, they can also be used to apply on body regions which can't be reached by hands, and will avoid abrasion or hurting the diseased skin. Hence, we also attempted to prepare sprays of the muds.

Preparation

Mud powder: 25 gms (passed through 500#, <25um size)

Methyl paraben: 0.1% w/w

Propyl paraben: 0.1% w/w

Distilled water: q.s.

Parabens were mixed in small quantity of water and added. The remaining water was added gradually, while stirring so that no agglomerates formed. The fluidity of spray was a little more than that of lotion. Instruction "Shake well before use" was labeled on it.

It was then filled in a spray bottle (fig.no.73) whose nozzle opening was approx. 0.7mm.

8.5.1. Optimization of water

Rheology of sprays was also measured in the same manner as pastes and flow was characterized by viscosity at 1 rpm. (Brookfield, RV model, spindle no.7). Kerala Black, Brown, Dwarka and Vadodara showed viscosity of 1,50,000, 1,70,000, 1,40,000, 1,60,000 cp at 1 rpm which was considered optimum for flow and the amount of water added to produce these viscosities respectively was considered optimum.

8.5.2 Evaluation

The sprays were evaluated for sedimentation and redispersibility as described earlier for lotions.

8.5.2.a Sedimentation volume

Sedimentation volume was 0.9 ± 0.02 for Kerala Black, 0.92 ± 0.03 for Kerala Brown, 0.87 ± 0.04 Dwarka, 0.89 ± 0.02 for Vadodara after 10 hrs, indicating the suspendability of the fine clays of the mud and the low rate of settling which is important for delivering uniform dosage from the spray bottle.

8.5.2.b Redispersibility:

It was observed that 4 to 5 forceful pendular motions (for all muds) were sufficient to redisperse the spray dispersions.

8.5.2.c. Spray pattern

The spray bottle was shaken gently and the spray lever pressed, to eject the dispersion from the spray nozzle, on the hand placed in vertical position one and a half feet away. To study the pattern, it was also sprayed on ceramic wall. Fig.no. 74 shows the pattern of one pressing but 6 to 7 pressings were needed to uniformly cover the area of 5 x 7 sq.cm.

Since the spray liquid is a suspension of high solid content, the spray pattern observed is not like that of a solution (mist like). Fig.no.74 and 75, show that the sprayed suspension did not trickle down from the vertically kept hand nor from the ceramic wall. This quality of it makes it more user friendly as it will allow the user to apply it on all body parts including those that he /she cannot access directly.

But the major drawback we observed was that the nozzle had to be washed thoroughly every time the suspension was sprayed. If the nozzle was not washed properly, then the opening used to clog, thereby preventing spraying of the suspension next time. As we could not find a solution to this practical problem and as it was difficult for the patient to clean the nozzle after every use, we could not provide these spray formulations to the patients during clinical studies.

8.5.2.d Microbial evaluation

Microbial evaluation test was performed in the same manner as in lotions and no growth was seen in the plates incubated at 28⁰ and 37⁰ C.



Fig. no 73 Spray bottle used for spray formulation



Fig.no 74 Spray pattern on hand (kept vertical)



Fig.no. 75 Spray pattern on ceramic wall

8.6 Clinical studies (Formulations)

Screening of mud for clinical studies

Comparisons of physicochemical data and other tests of all four soils were done in an effort to correlate this data with its clinical efficacy, one of the objectives of our study. It is shown in table no.55.

Table no:55 Summary of characteristics of four muds

Sr.No	Characteristics	Kerala Black	Kerala Brown	Dwarka	Vadodara
01	Clay content	38%	42%	26%	18%
02	Swelling index	60%	120%	33%	33%
03	pH	5.89	7.39	7.34	7.79
04	Electrical conductivity	9.5±0.04mS	4.97±0.09	0.95±0.02	1.51±0.08
05	Organic carbon (chemical digestion method)	0.032±0.04	0.127±0.027	0.005±0.059	0.029±0.005
06	Ca content by AAS	0.035%	0.004%	0.016%	<0.5ppm
07	Mg content by AAS	0.22%	0.004%	0.007%	<0.4ppm
08	Ca content by ICP-AES	27.888%	4.81%	10.59%	6.94%
09	Mg content by ICP-AES	45.61%	10.37%	5.94%	2.79%

10	Humic acid content	0.182±0.01 4%	0.122±0.022 %	0.086±0.000 6	0.076±0.008
11	Skin penetration of Humic acid	1.899%	1.636%	Not detectable	Not detectable
12	Skin penetration of Magnesium	10.89%	83.22%	40.06%	40.86%
13	Skin penetration of Calcium	23.09%	45.53%	30.78%	44.66%
14	Preliminary clinical testing(t value)	1.95	2.99	2.35	2.86
15	Formulations(paste) Spreadability cms	4.2±0.2	4.9±0.1	4.5±0.2	3.9±0.3
16	Lotions Zeta potential	-14.1	-26.6	-16.4	-22.7
17	Sedimentation volume	0.94±0.01	0.95±0.03	0.9±0.03	0.92 ±0.02
18	Redispersibility no.of inversions	6	4	5	4

(1) Amongst the four, Kerala Brown was richest in clay, which meant it could adsorb large amount of ions.

(2) Moreover Kerala Brown had neutral pH (7.39), (pH of Kerala Black was 5.89). During clinical trials, it was observed that some patients (acne) who were very sensitive to sun heat, and were given Kerala Black to apply, did complain about slight but tolerable irritation of skin.

(3) Kerala Brown had highest amount of soluble organic carbon (chemical digestion method) concentration and as organic carbon comprises of many therapeutically active components, it was possibly thought to be more effective than other three muds.

(4) Highest swelling index of Kerala Brown indicated high mobility of elements. This was observed during diffusion studies as shown in table no: 34. Kerala Brown had maximum skin penetration of magnesium and calcium.

(4) Kerala Brown had reasonably good concentration as well as penetration of humic acid.

(5) Clinical evaluation of muds on psoriasis, eczema and acne showed that Brown was the best mud.

(6) Lotion of Kerala Brown had highest negative zeta potential, and minimum inversions needed for redispersion.

(7) Spreadability of Kerala Brown paste was highest.

Thus, to summarize, all the **physicochemical observations** helped us to conclude that amongst the four muds, **Kerala Brown** would be most effective in treating psoriasis, eczema and acne. This inference was further confirmed by results of preliminary clinical studies. Hence, for studying and comparing the **efficacy** of the **prepared formulations**, , we took up only **Kerala Brown formulations** for clinical studies.

Out of the total five formulations prepared, four formulations were clinically tried on patients suffering from psoriasis, eczema and acne. Due to practical problem of clogging during the use of spray, we did not use it for clinical studies. These studies were conducted at Dahod Government General Hospital, Dahod, Dist. Panchmahal, by a team of Dermatologists. In all 48 patients were studied. **Four patients were used for each formulation and each disease.**

Protocol of the clinical study remained the same as in preliminary clinical study(section 7.1 of the text) except for the method of application of the formulations. Evaluation of the disease symptoms was also the same as in preliminary clinical studies.

Patients were asked to apply the formulations on the affected part. Paste was to be applied gently and not to be rubbed. Lotion was to be gently applied by hand. Powder was to be uniformly sprinkled on the affected part or to be applied with puff. Mud Pack, was, to be held with the overlapping plastic from the middle, the patch was to be opened only upto the cloth portion, little water sprinkled on it to wet it and then stuck on the affected part.

All these formulations were applied once in 24 hrs. Paste, lotion and mud pack was removed after 30 minutes because they dried within that period and since they formed a visible layer of mud on the body, it would not be cosmetically accepted by the patient. However, the powder was not removed physically as it was not visible on the body and hence was allowed to remain on the body, with the skin moisture helping its retention.

The patients were called for follow-up visits after 2 weeks and evaluated. For psoriasis patients, PASI (Psoriasis Area Severity Index) score was determined by evaluating reduction in erythema, desquamation, scaling and itching. Eczema patients were evaluated for erythema, itching and redness and Acne patients for no. and intensity of comedones their size and redness.



Fig.no.76 Psoriasis before treatment (paste)



Fig. no. 77 Psoriasis after treatment (paste)



Fig.no. 78 Eczema before treatment (lotion)



Fig.no.79 Eczema after treatment (lotion)



Fig.no. 80 Psoriasis before treatment (mud compress)



Fig. no . 81 Psoriasis after treatment (mud compress)



Fig.no. 82 Acne before treatment (powder)



Fig.no. 83 Acne after treatment (powder)

Results and discussion

The results of effect of various formulations on the disease symptoms are shown in table no. 56.

Table no: 56 % improvement in disease symptoms after application of formulations (2weeks)

Disease	Paste	Lotion	Mud compress	Powder
Psoriasis	52±4.5	67±2.5	55±4.4	50±2.4
Eczema	60±3.9	74±4.8	-	62±3.6
Acne	55±5.2	65±3.7	61±4.5	70±4.2

Depending upon the intensity and % of area affected, there was variable reduction in the improvement scores (table no. 56) but in general, there was more than 50% improvement in all disease symptoms. 50% improvement in symptoms in psoriasis is especially a clinically significant end point as described by Carlin (Carlin CS 2005).

8.6.1. Pastes

Psoriasis:

There was approx. 50% reduction in symptoms in approx. 2 weeks.. Reduction in itching was the first sign of improvement. Then gradually the scales reduced, but the plaque took a longer time, (approx.12 weeks), depending upon the intensity of the disease, to reduce. Out of four patients, one patient who had a disease history of 6 yrs and had 30% body surface covered with lesions was monitored for six months with continuous mud (paste) therapy. His lesions disappeared within 3 months and there was, no re-eruption upto 6 months. In fig. no. 76 and 77 it can be observed

that the scaling was removed and the boundary of the lesion improved prior to the central region after 15 days of the treatment.

Eczema: 60% of the lesion was recovered within 2 weeks and there was no complaint of irritation or itching.

Acne: Those who had comedones on cheeks, improved faster than those on forehead. The softness & reduced thickness and higher permeability of skin of cheeks may be responsible for this difference.

8.6.2. Lotions

Patients who applied lotions had experienced sense of softness and better feel than paste due to their ease of application, and fluidity. They also expressed cooling sensation due to which they felt calm, which may have indirectly reduced their stress and hence helped in disease control.

Psoriasis: 67% recovery was observed in psoriasis patients. Water content being more in lotions than in pastes, diffusion of mud components through mud will be faster, resulting in faster penetration of minerals into skin which is reflected in greater percentage of improvement in disease symptoms. Moreover as it contained about 50% solid content, adsorption of components from skin on soil was also expected to be effective.

Eczema: Improvement in eczema was around 74%. It could be observed from the fig.no. 78 & 79 that the skin lesion was healing and the clear cut boundary of eczematous patch was in the process of resolving and was gradually assuming normal skin texture.

Acne: 65% improvement was noticed in acne patients using lotion. Lotion was more favoured on face than paste because the cooling effect on face immediately reduced stress and helped in recovery. It should be noted that like psoriasis and eczema, stress is one of the trigger factor of acne also.

Due to more water content in lotion as compared to paste, it dissolves organic and inorganic compounds of soil and permits their diffusion, (Lai TM 1962), faster than in paste. Diffusion of compounds from lotions into the skin and from skin to the soil is faster in aqueous environment and so diffusion is more in presence of water. Probably this may be the reason why lotions are more effective than paste as seen clinically.

8.6.3. Mud Compress (mud pack)

Mud compresses were tested on patients of psoriasis and acne only. It was difficult to find patients whose eczematous lesions matched to the size of the prepared mud packs. Psoriasis patients showed 55% improvement while acne patients showed 61% improvement. For acne, it was easy to get patients because the comedones are mostly concentrated on the cheek and the forehead. But applying pack on cheek was not acceptable because pulling of adhesive tape from soft skin, for removal, was a bit painful. Hence, mud packs were used by patients with acne on the forehead as it was convenient to apply there. When applied on forehead for acne patients, the results were very good due to combination of two effects: **adsorption** due to very high content of paste and increased **diffusion** due to occlusion effect. The **cooling effect** lasted for longer time than lotions and so gave a pleasant feeling which helped reduce stress.

8.6.4. Powder

Powder was better accepted because it was convenient to apply and could be kept on the affected part for long without any feeling of uneasiness and without affecting aesthetic appearance of the skin. The powder used for application was very fine, less than 25µm size, so there are very less chances that it may be rubbed off by friction. Moreover, it adheres to the skin due to its (skin's) moisture. However, application of powder caused dryness of skin and hence was not preferred by patients in winter.

Adsorption of organic molecules on soil is accompanied by desorption of Type II water from the colloid surface (McBride MN 1975, White JL 1972). This results in a net entropy gain for the system, which is reflected in rise of temperature as time passes, after application of mud formulations like paste, lotions and mud compress but not powder.

Psoriasis:

Psoriasis patients experienced a sense of dryness after applying powder and so many times it was recommended that they apply a thin layer of oil (coconut oil or til oil) on the body and then apply the powder. The improvement was slow, i.e. 50% in 2 weeks time. Moreover they did not prefer in winter because as it is, in winter the skin becomes dry and powder increased the dryness.

Eczema:

Eczema patients also showed reasonable improvement (62% improvement) in 2 weeks. The problem of dryness persisted in eczema also but it was not so strong enough to avoid usage of powder in winter.

Acne

It is quite evident from fig. no. 82 and 83 that acne patients had 70% improvement in 2 weeks. Powder may have adsorbed the sebum secretion from the skin and prevented it from being spread on the entire face due to perspiration. Moreover, unlike cosmetic face powder, the mud powder did not show off on the face in presence of perspiration. Amongst the four formulations, it was the most accepted by acne patients.

Since the chemistry of clay surfaces is different in wet and dry environments, (more adsorbing nature when dry) the results obtained in one system may not apply in the other even with the same organic compounds (Mortland, M.M. 1970). So adsorption phenomenon of compounds from skin to soil, will be different when mud will be used as paste, lotion, or spray and dry powder. When dry soil (powder

formulation) is applied on oily skin (acne), it keeps the skin dry throughout the day. It may be due to this that mud shows its therapeutic activity in wet as well as dry condition differently, i.e. more adsorbing in dry condition.

Thus, the clinical data of formulations led to the inference that lotion was more effective on psoriasis and eczema while powder was more effective on acne patients. The probable reason for this different effect may be because psoriasis and eczema get ameliorated due to dryness of the skin which is caused by powder, while acne (one of the cause being excessive sebum) gets more relieved by it because it (powder) adsorbs the skin sebum and keeps the face dry.

The probable mechanism of actions of mud may be attributed to multiple actions like (1) activity exerted through diffusion of its constituents (2) adsorption of skin constituents (3) action due to its cooling property (4) actions due to its occlusion property and other activities (antimicrobial). We have seen earlier that mud is very rich in organic and inorganic components and that pharmacological actions of calcium, magnesium, sodium, potassium, (inorganic) and humic acid (organic) is well established in psoriasis, eczema and acne. Adsorption of carbon compounds from diseased lesions has been observed.

In **pastes**, which has very high solid content, the predominance of activity may be in the order: **adsorption mechanism > due to constituents > cooling property**.

In **lotions**, due to high water content compared to pastes, the predominance may be in the order: **diffusion of its constituents > cooling property > adsorption mechanism**.

In **mud-compress**, due to occlusion created by the adhesive tape, the predominance may be in the order: **occlusion property > adsorption mechanism > diffusion of constituents > cooling property**.

In **powder**, due to absence of water, the predominance of activity may be in the order: **adsorption mechanism > diffusion of constituents**.

Thus, we can conclude that we could successfully formulate at least four mud formulations which can be used therapeutically i.e. pastes, lotions, mud compresses and powder. Although a spray formulation was also developed, the problem of clogging of the spray orifice limited its practical utility.

Clinical trials using the developed formulations showed more than 50% improvement in disease symptoms in all diseases, with maximum improvement shown upto 74% by lotion for eczema patients.

Hence, we can conclusively say that we were successful in fulfilling our objectives of preparing clinically effective, patient friendly mud formulations for the treatment of various skin disorders like psoriasis, eczema and acne.

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