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FORMULATION AND EVALUATION OF GEL OF DIMETHYL DISULFIDE-SILVER COMPLEX

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ABSTRACT

During the past several years a number of complexes of transition metals have been reported in which the central metal ions have been coordinated by aromatic or unsaturated compounds containing sulfur donor atoms. Dimethyldisulphide which has been reported to possess antibacterial, as well as antifungal activity is one of the main constituents of *Allium sativum* (Garlic). In the present study dimethyldisulphide has been complexed with silver metal. The complex so formed has been characterized by spectroscopic methods, further the complex has been formulated into a gel. Different gels were prepared with varying concentration of Carbopol 934. Minimum inhibitory concentration (MIC) was calculated using cup-plate method. Gels were further evaluated for physical properties like color, pH, spreadability, consistency, diffusion, stability and viscosity. Evaluation of gel was further done against *Staphylococcus aureus (ATCC 6538),* and *Pseudomonas aeruginosa (ATCC 9027)*. The gel showed promising antibacterial activity against microorganisms used for study. The Gel was stable at room temperature.

Keywords: *Dimethylsulphide; Garlic; Silvercomplex; Gel*

INTRODUCTION

Garlic (*Allium sativum*), a member of the lily family, is a perennial plant that is cultivated worldwide. Garlic is used for different purposes such as a palliative for the heat of the sun in field labor, heart disease (including atherosclerosis, high cholesterol and high blood pressure), cancer and cardiovascular effects of garlic. It is also reported to help to regulate blood sugar levels and also shows antimicrobial activity. In modern naturopathy, garlic is used in the treatment for intestinal worms and other intestinal parasites, both orally and as an anal suppository.[1-2] Garlic cloves are used as a remedy for infectious diseases (especially chest problems), digestive disorders, and fungal infections such as thrush, candidiasis, aspergillosis and cryptococcosis.[3-5] Garlic is mentioned as remedy for skin diseases in Ayurveda and it is chosen for antibacterial activity because unlike antibiotic there is no development of resistance in case of garlic. Garlic is famous for its characteristic odor, arising from allicin and other oil soluble sulphur components. Typical volatiles in crushed garlic and garlic essential oil include diallyl sulphide (DAS, diallyl disulphide (DADS), dimethyldisulphide(DMDS) , diallyl trisulphide, methyl allyl trisulphide, 2-vinyl 1,3 dithiin, 3- vinyl 1,2 dithiin (6) and E,Z –ajoene.[6]

Transition metals like silver have been used for years as antimicrobial agents. This activity of silver has started the development of metal based drugs with promising pharmacological action and may offer unique therapeutic opportunities. Silver has low toxicity as compared to other transition metals. Silver nitrate is still given to infants to prevent the development of ophthalmia neonatorum. Complexes containing silver (I) are clearly the most abundant of the silver complexes. What makes the silver (I) cation remarkable is its ability to coordinate in a range of geometries, from linear through to octahedral and in some cases, even higher geometric orders. Silver (I) complexes with sulfur containing ligands exhibit a wide range of applications in medicine. One of the most commonly used compounds of silver is silver (I) sulfadiazine; it is used to treat severe burns to prevent bacterial infections [7]. There is no report on a complex of dimetyldisulphide and silver. Moreover, the incorporation of silver metal into dimethyldisulphide enhances the biological activity of the ligand and decreases the cytotoxic effects of both the metal ion and ligand on the host. Considering this, the present study was initiated with synthesis, characterization and antimicrobial screening of the complex. The gel was prepared for topical drug delivery by using different concentrations of Carbopol 934. The gels so prepared were evaluated for their appearance, pH, spreadability, antimicrobial activity, permeation and also for its stability studies.

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DIMETHYL DISULFIDE- SILVER COMPLEX GEL

EXPERIMENTAL

All chemicals were purchased from Aldrich and Merck chemicals, Mumbai (India), and were used without further purification. Melting points were determined in open capillaries using a decibel melting point apparatus and are uncorrected. Formation of compound was routinely checked by TLC using Silica G, and spots were exposed to iodine vapor for visualization.
The IR spectra in KBr were recorded on a Perkin - Elmer FT-IR spectrometer.

^{1}H NMR spectra was obtained in deuterated dimethylsulfoxide (CD3)2SO) on a Bruker Avance spectrometer and Mass spectra on LCMS Agilent technologies, model 6520.

Synthesis of complex

A solution of silver nitrate (5 mmol) in hot ethanol (20 mL) was mixed with hot ethanolic solution of the dimethyldisulphide (5 mmol), maintaining the pH of solution at 10. The resulting solution was refluxed for 4 hours on a water bath. On cooling the contents, the colored solid separated out. The product was filtered, washed with cold 50% ethanol, and dried in oven at 100°C.

Molecular formula: $C_2H_6AgS_2$
Solubility: soluble in EtOH, MeOH and DMSO
Anal. Calc.: C, 11.89; H, 2.99; S 31.7%.
Anal. Found: C, 11.80; H, 2.66; S 30.8 %.
IR (cm^{-1}, KBr): 1384(CH3-S asym), 682 (C-S-C stretch);
^{1}H NMR (ä, DMSO):2.30~2.32(s , 6H, CH_3)
Mass spectra: m/z peak found to be: 201 [(L) Ag] and 94 [L]

Formulation of gel

Different quantities of carbopol were soaked in distilled water containing methyl paraben 0.15% and propyl paraben 0.05% and 2% Glycerine for 12 hours and then dispersed by agitating at approximately 600-700 rpm with the aid of a mechanical stirrer to get a smooth dispersion .The drug was dispersed in polyethylene glycol and sonicated using probe sonicator (Bandelin Sonopuls) for 20 minutes at 50% efficiency . This was added to polymer dispersion and stirred for 10 minutes. The pH of the formulation was adjusted to 6.4 by adding triethanolamine and then stirred at 300rpm for 10 minutes. (Table 1)

Table 1 : *Formulation composition of gels*

Sl.	Ingredients	Quantity of ingredients (%)								
		Batch number code								
		A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9
1	Carbopol 934	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.25	2.5
2	Drug(complex)	1	1	1	1	1	1	1	1	1
3	Polyethylene glycol 400	5	5	5	5	5	5	5	5	5
4	Methylparaben	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
5	Propylparaben	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
6	Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
7	Water (up to	100	100	100	100	100	100	100	100	100

Evaluation of gel

The prepared gels were evaluated for the following parameters. Results are given in (Table 2)

Table 2 : *Evaluation parameter of gels*

Sl.	Batch code	Clarity	pH	Homogeneity	Spreadability (g cm/sec)	Drug content (%)	Extrudability
1	A_1	+	6.5	Good	26.90	97.2	-
2	A_2	+	6.5	Good	25.71	97.5	-
3	A_3	+	6.6	Good	23.96	98.6	-
4	A_4	+	6.5	Good	23.10	98.5	-
5	A_5	++	6.4	Good	22.20	98.7	+
6	A_6	++	6.5	Good	20.75	98.6	+
7	A_7	++	6.5	Good	19.68	99.4	++
8	A_8	++	6.4	Good	19.12	99.3	++
9	A_9	+++	6.5	Good	18.37	99.6	+++

+ : Poor ++ : Good +++ : Very Good

pH

The pH of various gel formulations was determined by using digital pH meter. (Systronics)

Spreadability

It was determined by wooden block and glass slide apparatus. Weights about 20g were added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed Slides.[8] Spreadability was then calculated by using the formula:
S = M.L / T

Where,

S = Spreadability

M = Weight tied to upper slide

L = Length of glass slide

T = Time taken to separate the slide completely from each other

Homogeneity

All gels were tested for homogeneity by visual inspection after the gels had been set in the container .They was tested for their appearance and presence of any aggregates.

Drug content

100mg of developed gel was taken and 100ml of phosphate buffer of pH 6.4 was added. The containing gel solution was then shaken for 2hours on mechanical shaker. This solution was filtered and estimated spectrophotometrically (Shimadzu 1700) at 230 nm using phosphate buffer (pH 6.4) as blank.[9]

As batch A_9 had best Homogeneity, Spreadability & Extrudability, it was further evaluated.

Studies with Optimized Gel A_9

Viscosity

Viscosity was determined at room temperature using Brookfield viscometer ((Brookfield DV-II + pro viscometer) and found to be 9236cps.

Particle size analysis

a. Zeta Sizing: Optimized gel A_9 was diluted with double distilled water & particle size determined with Zeta sizer using Malvern instruments (IIT Delhi) (Fig.1)

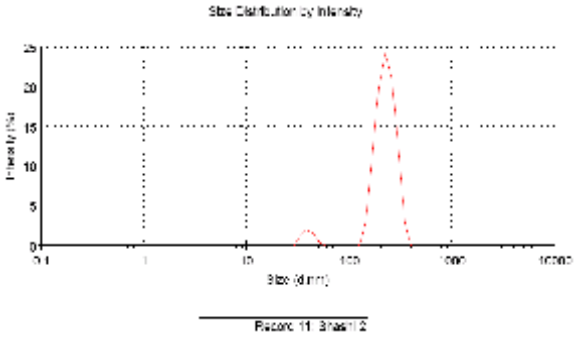

Fig.1: *Particle size analysis*

b. Scanning Electron Microscopy:

SEM analysis of prepared gel was done at IIT Delhi. (Fig.2)

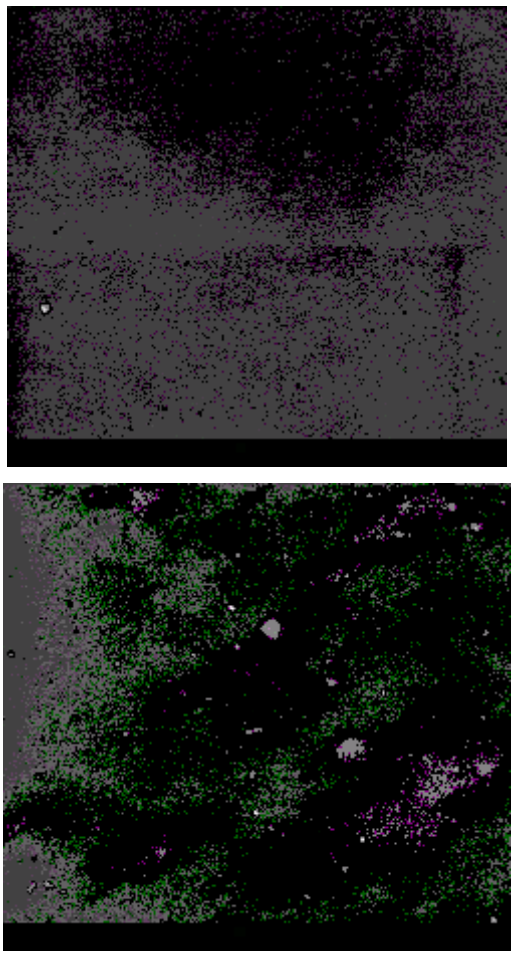

Fig. 2 : *Scanning Electron Microscopy*

Stability Studies

The selected formulation A_9 was subjected to stability studies by keeping at ambient temperature for three months. Next it was analyzed for change in appearance, pH or drug content[10] (Table 3)

Table 3 : *Stability study of optimized gel*

Months	ppearance	pH	Drug Content(%)
0	Clear	6.5	99.6
1	Clear	6.4	99.4
2	Clear	6.4	99.2
3	Clear	6.4	99.1

Permeability studies

The pretreated skin of albino mice was used in franz diffusion cell. The skin was clamped between the donor and the receptor chamber of vertical diffusion cell with an effective diffusion area of 1.767 cm^2 and a cell volume of 18 ml. The receptor chambers were filled with freshly prepared phosphate buffer pH 6.4. The diffusion cells were maintained at 37 °C using a re-circulating water bath and the fluid in the receptor chambers was stirred continuously at 300 rpm. The formulation (1 g) was gently placed in the donor chambers. The sample at predetermined intervals were withdrawn and replaced by equal volume of fresh fluid. The samples withdrawn were spectrophotometrically estimated at 230 nm against their respective blank.[11] (Table 4, Figure 3)

Table 4 : *Permeability studies of optimized gel*

Sl.No.	Time (hrs)	Absorbance	Conc (µg/ml)	Amount of drug in 18ml	Cumulative amount of drug permeated		% drug permeated
					in 1ml	in 18 ml	
1.	1	0.042	102.439	1843.90	0	1843.90	18.43
2.	2	0.048	109.756	197.580	212.19	2187.79	21.87
3.	4	0.062	151.2	2721.95	363.39	3085.34	30.8
4.	6	0.071	173.170	3117.07	536.56	3653.63	36.5
5.	8	0.071	173.170	3117.07	709.730	3826.80	38.2

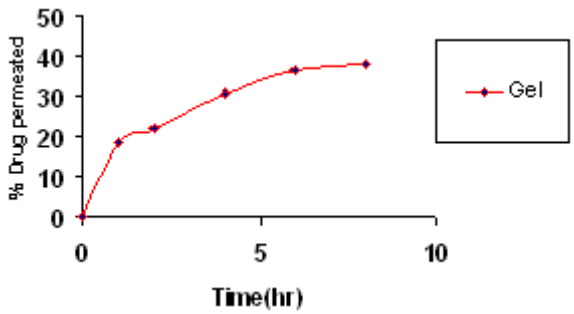

Fig 3 : *Percentage Drug permeation*

Antimicrobial study of optimized gel

Evaluation of Antibacterial activity

The evaluation of antimicrobial activity of gel formulation A_9 was done by *in vitro* methods by agar diffusion technique using *Staphylococcus aureus*

(ATCC 6538) and Pseudomonas aeruginosa (ATCC 9027).

Agar diffusion method
Principle
The agar diffusion method depends upon diffusion of antibiotic from a vertical cylinder through a solidified agar layer in a Petri plate to an extent such that the growth of the added microorganisms is prevented entirely in a zone around the cylinder containing a solution of the antibiotic.[12]

Method
To 100 ml of previously molten nutrient agar media 0.4 ml of the inocculum was added and shaken. The material was next poured into sterile plates, allowed to solidifying taking care that the thickness of layer is uniform. It was next, incubated for 24 hours at 37°C and then the zone of inhibition was measured. Results are presented in Table 5.

Table 5 : *Antibacterial activity of gel*

S.N	ORGANISM	ZONE OF INHIBITION (mm)				
		DMDS	PURE COMPLEX	MARKETED FORMULATION		PREPARED FORMULATION A_9
				GEL	CREAM	
1	*Staphylococcus aureus*	16	18	17	16	17
2	*Pseudomonas aeruginosa*	17	20	15	16	18

Concentration 100 µg/ml
GEL: 10% PLACENTREX GEL
CREAM: SILVERSULFADIAZINE

RESULTS AND DISCUSSION

The decrease of the δ(CS) frequencies in the I.R spectra of the complex indicates S-coordination of the ligands. The IR spectra of the metal complexes showed that the band at 1429 cm^{-1} assigned to the $\delta(CH_3\text{-}S)$ vibration of the ligand (Dimethyldisulphide) as shifted to lower frequency after complexation to 1384 cm^{-1}. The presence of sharp singlet for the proton clearly indicates that the magnetic environment is equivalent for all such protons, suggesting the presence of a planar ligand in the complex. The mass spectroscopy was performed on complex to determine its molecular weight and fragmentation pattern. Complex shows peak corresponding to molecular ion(M^+) and free ligand(L^+), which confirms the formation of the complex. The melting point of complex was found to be 180 °C. The solubility of drug was soluble in methanol, ethanol and in water. The pH value of developed formulation A_9 was 6.4. The value of spreadability indicates that the gel is easily spreadable by small amount of shear. Spreadability of formulated gel A_9 was 18.37gcm/sec. The extrudability reflects the capacity of the gel, to get ejected in uniform and desired quantity when the tube is squeezed. Hence, the extrudability of A_9 formulation was the best as compared with other formulation. All developed gel showed good homogeneity with absence of lumps. The formulated A_9 preparation was much clear and transparent as compared to other formulation Thus observations indicates acceptability of this gel for topical use. During the accelerated stability studies the appearance was clear and no significant variation in pH was observed and drug content is 99.2 % in A_9 formulation after 3 months. In vitro Permeability study showed that permeation, after 8 hours was 38.2%.

Synthesized complex shows significant antimicrobial activity against microorganisms as compared to dimethyldisulphide. The gel showed significant activity against skin pathogens when compared to the standard drug placentrex gel 10% and silversulphadiazine cream. The gel base does not show any antimicrobial activity.

CONCLUSION

Gel with dimethyldisulphide- silver complex was formulated using Carbopol 934. Gel with Carbopol 934 shows good physicochemical properties. The antimicrobial activity of this gel formulation is comparable to standard (placentrex gel and silversulphadiazine) .The result suggests the feasibilility of gel for antimicrobial activity. Thus an observation indicates acceptability of this gel for topical use.

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REFERENCES

1. Sanchez-Moreno C. Food SciTech Int. 2002; 8: 121-137.
2. Kumaran A , Karunakaran J. Food Chemistry. 2006; 97(1):109-114.
3. Banerjee SK , Maulik S K. 2002; 1:4.
4. Heinrich P, Lawson L D. Garlic: The science and therapeutic application of allium sativum L. and related species Williams and Wilkins, 2nd edition, 1996 ; p 135-137.
5. http://www.gourmetgarlicgardens.com/chemistry.htm.
6. Fenwick GR, Hanley AB. Food Sci. Nutr. 1985; 22:273-377.
7. Clarke MJ. Coordination Chem. 2003; 236: 299-233.
8. Gupta GD, Gound RS. Ind J Pharm Sci. 1999; 61: 229-234.

9. Sera UV, Ramana MV. The Indian Pharmacist. 2006; 73:356-360.

10. ICH Harmonized Tripartite Guidelines, Stability Testing of New Drug Substances and Products, ICH Committee, 8, 2003.

11. Sahoo SK, Samal AR. The Indian Pharmacist. 2006: 5(46); 73. 1972; 78:216-218.

12. Spooner DF and Sykar G. Laboratory assessment of Antibacterial activity. In methods in Microbiology. Academic Press, London and New York, 1972; 78:216-218.