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Formulation, development and evaluation of clindamycin phosphate microspheres for topical delivery system

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Abstract
The aim of the present study was to formulate topical microspheres based delivery system containing Clindamycin for treatment of infections. The microsphere was prepared by double emulsion solvent diffusion method. Then after the optimized batches incorporated into gel base. Clindamycin phosphate is lincosamide antibiotic and is used mainly for treatment of acne. The microspheres of the optimum batch (N5) exhibited 79.1% drug entrapment efficiency, mean particle size of 310 µm and 94.96% release at 8 hour. An appropriate balance between the levels of the polymer (EC) and stirring speed was imperative to acquire maximum drug entrapment efficiency, control release of the drug, and adequate particle size. The optimize batch was further used for antimicrobial study and skin irritation study and also compared with marketed gel clindawel. Antimicrobial study was performed on nutrient agar media for *S.epidermidis* and agar media for *P. acne* because of both of microbes responsible for production of acne. The zone of inhibition of formulated gel against *S.epidermidis* and *P.acne* microbes was greater than marketed gel.

Keywords: formulation, evaluation, clindamycin, microspheres, topical, delivery system

1. Introduction
Microspheres are defined as solid spherical particles containing dispersed drug in either solution or microcrystalline form. They are ranging in size from 1 to 1000 micrometer. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 micrometer [1].

Double emulsion technique involves the formation of the multiple emulsions or the double emuulsion of type w/o/w and is best suited to the water soluble drugs, peptides, proteins. The aqueous protein solution is dispersed in a lipophilic organic continuous phase which is generally consisted of polymer solution that eventually encapsulates protein contained in dispersed aqueous phase. The primary emulsion is then subjected to the homogenization before addition to aqueous soolution of PVA which results in formation of double emulsion and is then subjected to soolvent removal by solvent evaporation maintaining the emulsion at reduced pressure or by stirring so that organic phase evaporates out [2, 5].

Clindamycin Phaosphate is chemically methyl-7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2pyrrolidinecarboxamido)-1-thio-L-threo-α-D-galacto-octopyranoside-2-(dihydrogen phosphate). It is used for the treatment of serious infections caused by susceptible anaerobic bacteria, including Bacteroides spp., Peptostreptococcus, anaerobic streptococci, Clostridium spp., and microaerophilic streptococci. Systemic/vaginal clindamycin inhibits protein synthesis of bacteria by binding to the 50S ribosomal subunits of the bacteria. Specifically, it binds primarily to the 23s RNA subunit. Topical clindamycin reduces free fatty acid concentrations on the skin and suppresses the growth of Propioni-bacterium acnes (*Corynebacterium acnes*), an anaerobe found in sebaceous glands and follicles [6-10].

2. Materials

Table 1: Materials used in the present Study

Clindamycin Phosphate	Gujarat Pharma Laboratory, Ahmedabad
Ethyl cellulose (16-22 cps) (EC)	Sword & Shield Pharmaceutical, Chhattral
Dichloromethane (DCM)	S. D. Fine Chemicals Ltd., Mumbai, India.
Ethanol (ETN)	S. D. Fine Chemicals Ltd., Mumbai, India.
Acetonitrile HPLC Grade	Finar Chemicals Ltd., Mumbai, India
Methanol HPLC Grade	Merck Ltd., Mumbai, India
Distille Water HPLC Grade	Finar Chemicals Ltd., Mumbai, India
Liquid Paraffin	S. D. Fine Chemicals Ltd., Mumbai, India.
Carbopol 934 P	S. D. Fine Chemicals Ltd., Mumbai, India.
Triethanolamine	S. D. Fine Chemicals Ltd., Mumbai, India.
Methy hydroxyl benzoate	S. D. Fine Chemicals Ltd., Mumbai, India.
Propylene glycol	S. D. Fine Chemicals Ltd., Mumbai, India.
Nutrient Agar	Himedia Lab. Pvt. Ltd. Mumbai
Blood agar	Himedia Lab. Pvt. Ltd. Mumbai

Table 2: Bacterial Strains used for antimicrobial study

Bacterial Strains	MTCC No.
Propionibacterium acnes	1951
Staphylococcus epidermidis	3615

3. Methods
Drug Excipients compatibility study
DSC spectra study
The possibility of any interaction between CLP, polymers and other excipients during the microencapsulation process was assessed by carrying out the thermal analysis on microspheres

using Differential Scanning Calorimetry. To carry out thermal analysis, Samples were accurately weighed and put into aluminum pans and then sealed with aluminum lids and calorimetry measurement was performed with DSC TA-60WS Shimadzu, Japan instrument, over the temperature range from 30 to 200 °Cat heating and cooling rates of 5 °C/min. The glass transition was reported as the point of inflection of specific heat increment. Samples of CLP, EC, and microspheres were analyzed in the aluminium pan and then their DSC spectra were recorded as shown in figure-1(a), (b), (c).

FT-IR spectra study

The Fourier Transform Infra-Red analysis was conducted for the analysis of drug polymer interaction and stability of drug during microencapsulation process. Fourier transform infra-red spectrum of pure CLP, Physical mixture and microspheres (formulation) were recorded using Fourier Transform Infrared Spectrophotometer 8400S Shimadzu, Japan. Sample preparation involved mixing the sample with potassium bromide (KBr), triturating in glass mortar and finally placing in the sample holder. The spectrum was scanned over a frequency range 4000-400 cm-1. Shown in figure-2(a),(b),(c).

Preparation of standard curve of Clindamycin Phosphate

CLP (10 mg) was dissolved in phosphate buffer pH 6.8 and volume was made up to 100 ml in 100 ml volumetric flask. This solution (100 µg /ml) was further diluted with phosphate buffer to obtain solution of 2 to 14 µg /ml. Peak area of each solution was measured at 210 nm using HPLC Shimadzu LC-2010. The standard curve was generated at 210nm. The results of standard curve preparation are shown in the Table 3.

Preparation of Ethylcellulose microspheres of Clindamycin Phosphate

All microspheres were prepared by the w/o/o double emulsion solvent diffusion method. Weighed amounts of Ethylcellulose and CLP were dissolved in 5 ml of a mixture of acetonitrile and dichloromethane (1:1). The initial w/o emulsion was formed by adding 2 ml of deionized water to the drug-polymer solution with constant stirring at 500 rpm for 5 min. The w/o primary emulsion was then slowly added to light liquid paraffin containing span 80 as a surfactant with constant stirring for 2 hrs. The resulting microspheres were separated by filtration, free from liquid paraffin by repeated washing with n-hexane (50 ml) and finally air dried over a period of 12h.

***In-vitro* Antimicrobial study using cup plat method**

Evaluation of *In-vitro* antimicrobial activity was carried out by cup plate method. The overnight grown culture of *P. acne* and s. epidermis was inoculated into the sterilized agar media plates containing blood agar media and nutrient agar media respectively. After solidification, wells were cut into the media by using sterile borer and fixed with 10 mg of the specimens to be tested using marketed gel and optimized microspheres containing gel. The plates were incubated at room temperature for 24hrs and the width of zone of inhibitions resulting after drug diffusion into media was measured.

4. Result and Discussion

The DSC thermo gram of CLP exhibited an exothermic peak at about 98.56 ºC corresponding to its melting point. But in thermal analysis, CLP-loaded microspheres show peak originating from CLP. Only slightly difference in peak of temp. From DSC spectra of CLP peak shown at 98.56ºC, and microspheres shown peak at 106 ºC, near to drug peak indicate that there is no significant change in DSC spectra of microspheres i.e., it is nearly same to that of plain compounds.

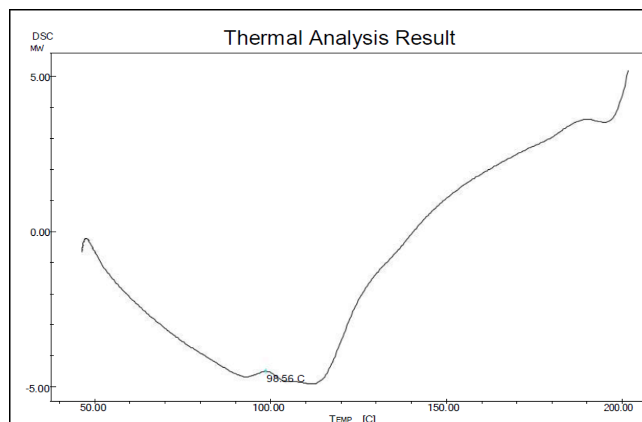

Fig 1(a): DSC spectra of Clindamycin Phosphate

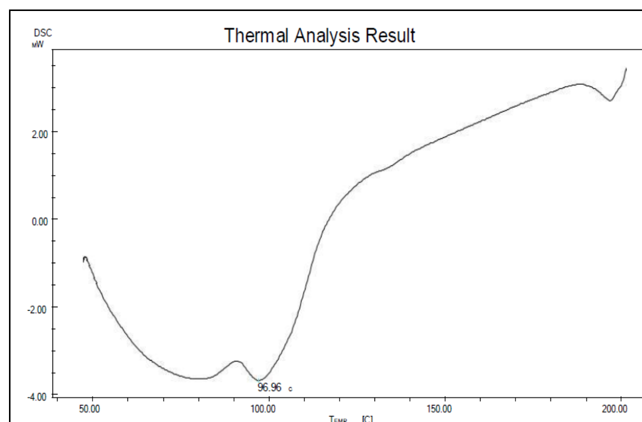

Fig 1(b): DSC spectra of Physical mixture

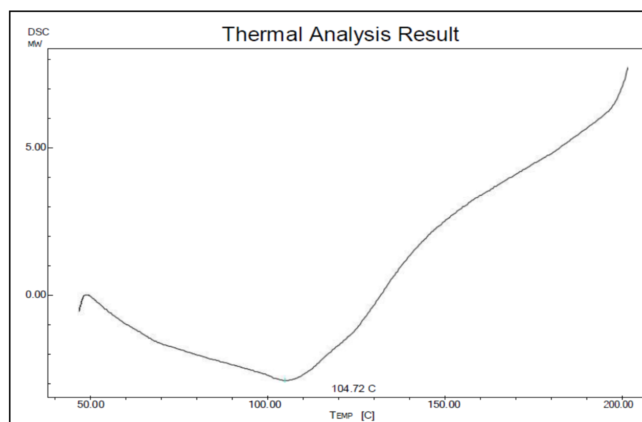

Fig 1(c): Thermal Analysis Result of Microspheres

From IR spectra of CLP, physical mixture of drug and polymer, and Microspheres it can be seen that there is no

significant change in IR spectra of microspheres i.e., it is nearly same to that of plain compounds. IR Spectra is shown in figure-2 (a), (b), (c). 2900 cm^{-1} peak show C-H str, 700 cm^{-1} peak show C- Cl str, 3200 cm^{-1} peak show N-H, 1180 cm^{-1} peak show -C-NH-, 3000 cm^{-1} peak show C-H str (pyridine).

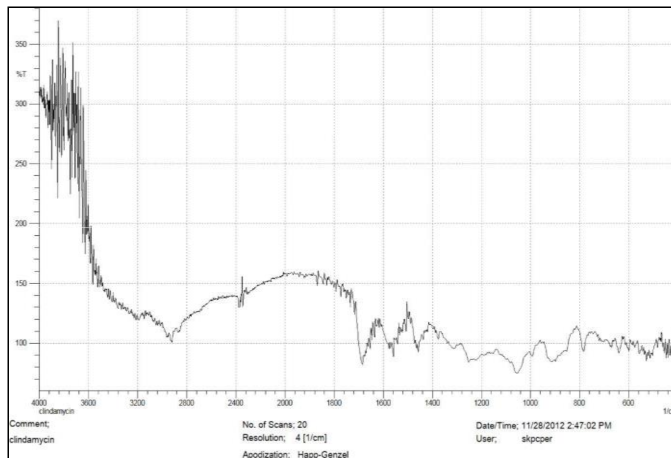

Fig 2(a): FT-IR of clindamycin phosphate

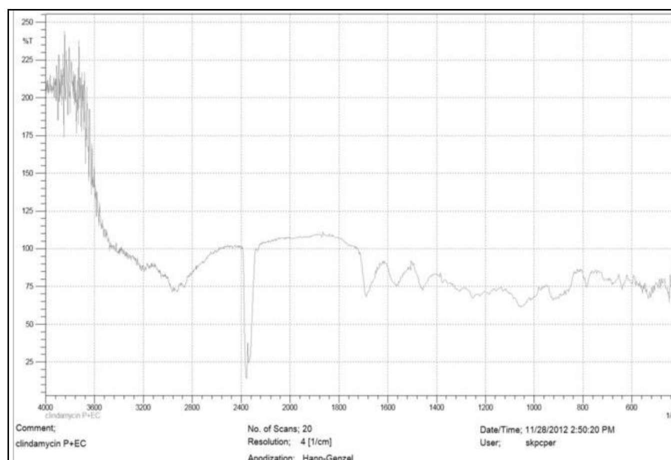

Fig 2(b): FT-IR of Physical mixtures

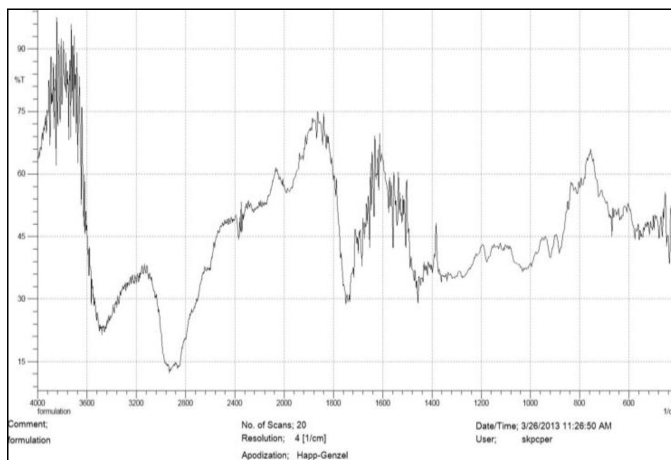

Fig 2(c): FT-IR of Microspheres

Standard curve was prepared according to procedure given in Chapter-6.2 the method obeys Beer's Law in the concentration range of 2 to 20 mcg/ml. The results of standard curve preparation are shown in table 3 and figure-3.

Table 3: Calibration curve of Clindamycin phosphate

Conc(µg/ml)	Area of peak			Average
2	260792	250790	250793	254125
6	286410	286409	286411	286410
10	307296	307298	307297	307297
14	329497	329495	329494	329495.33
18	351205	351198	351212	351205

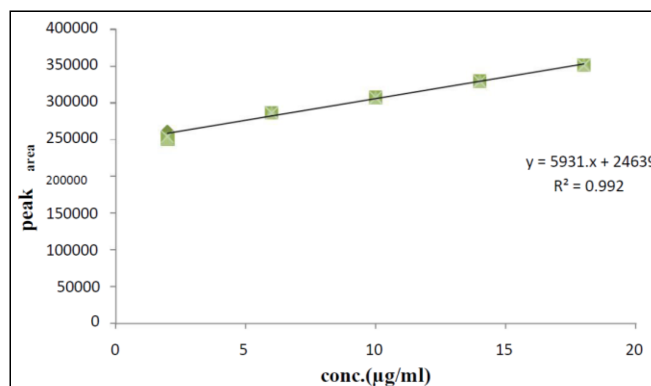

Fig 3: Standard calibration curve of clindamycin phosphate in pH 6.8.

In the preliminary studies, CLP microspheres were prepared by double emulsion solvent diffusion method. ACN and DCM was used for preparation of microspheres, in which polymer easily dissolve. The different ratio of ACN: DCM was found to have significant effect on drug entrapment for formulations P1 to P3, as a volume of DCM increased entrapment of drug decreases. Same as a volume of ACN increased entrapment of drug also decreased. Therefore, equal ratio of ACN: DCM was selected because of by using equal ratio (1:1), entrapment of drug increased. As a concentration of surfactant span 80 increased for preparation of CLP microspheres the entrapment of drug decreased for formulations P4 to P6. So, 0.5% of span 80 as a surfactant was selected for preparation of CLP microspheres because of entrapment efficiency of drug was more than 1% and 1.5% concentration of surfactant. As a low volume (50 ml) of processing medium was used for preparation of CLP microspheres decreased entrapment of drug for formulations P7 to P9. As a volume (150 ml) of light liquid paraffin was increased entrapment of drug also decreased. So, after optimization volume of LLP increased, the entrapment of drug decreased. So, 100 ml volume of LLP was selected for preparation of CLP microspheres.

Table 4: Optimized Batches

Batch Code	% Yield	% EE	% CDR	Mean particle size
N1	70.81	62.28	79.61	367.3
N2	73.86	64.40	74.8	376.9
N3	75.1	67.87	69.2	423.5
N4	81.92	70.42	89.80	302
N5	85.67	79.10	94.96	310
N6	87.83	72.40	84.37	318
N7	74.50	52.16	78.29	348
N8	77.9	55.20	74.20	356
N9	80.4	57.40	71.73	337

In vitro diffusion studies of CLP microspheres gel were performed in phosphate buffer pH 6.8 for 8 hrs using Franz

diffusion cell. It was found that% cumulative drug release from gel formulation N1 to N9 was 69.2% to 94.31% within 8 hrs. It was show that as a concentration of EC increased the drug release decreased. There was no any burst effect seen from release of drug. Within 8 hrs N5 batch was show 94.31% drug release.

Table 5: Comparison of cumulative% drug release with marketed gel

Time(hrs)	Formulated gel	Marketed gel
0	0.00	0
1	14.51	14
2	24.78	21.78
3	35.40	39.7
4	46.30	44.9
5	57.93	51.6
6	70.12	62.78
7	82.12	76
8	94.31	80.7

Microbial strain of *S. epidermidis* and *P. acne* both microbes responsible for creation of acne. So, it is necessary to check activity of formulated gel and marketed gel against *S. epidermidis* and *P. acne*.

Table 6. Antimicrobial Study

Microbial Strain	Marketed gel				Formulated gel			
	Zone of inhibition (mm)							
	I	II	III	Avg	I	II	III	Avg
S.epidermidis	35	36	36	35	60	55	59	58
P.acne	38	39	38	38	41	40	43	41

5. Conclusion

The present study reports the development of drug-loaded microspheres of clindamycin phosphate incorporated into gel base for topical delivery. The IR spectra revealed that, there was no interaction between polymers and drug. All polymers used were compatible with drug. The yield of microspheres were depended on the diffusion rate of acetonitrile and Dichloromethane and also on the concentration of EC, the mixing ratio of components in the organic phase; stirring speed. These all parameters were also affected on particle size and entrapment of drug into Microspheres. EC having good encapsulation efficiency and drug release retarding ability in nontoxic nature. Therefore, various concentration of EC and rotation speed was selected and optimization carried out for% entrapment of drug, mean particle size and% cumulative drug release study by diffusion study. After incorporation of microspheres into gel base, the all evaluated parameters of formulated gel were compared with marketed gel. It was show that% cumulative drug release from gel was follow zero order kinetic means provide control drug release but marketed gel was show 80.7% cumulative drug release after 8 hrs in compared to formulated gel was show 94.96% cumulative drug release after 8 hrs. It was found that there was no any skin irritation found on rat skin by applying formulated gel as well as marketed gel.

6. References

1. Akhand K, Samnani A, Panday G, Dubey BK. Microspheres as a novel drug delivery sysytem: a review, world journal of pharmacy and pharmaceutical sciences. 2012; 1(1):34-44.
2. Chawla C, Gupta P, Koradia V, Bansal AK. Gastroretention A Means to Address Regional Variability in intestinal drug Absorption. Pharmaceutical technology. 2003; 27(2):50-68.
3. Hickey AJ, Fults K, Pillai RS. Use of particle morphology to influence delivery of drugs from dry powder aerosols. J Bio pharm Sci. 1992; 3:107-113.
4. Jain NK. Controlled Novel Drug Delivery. Ist Eds CBS Publishers and Distributors, New Delhi, 2002, 236-255.
5. Jain NK. Progress in Controlled and Novel Drug Delivery Systems. 1st Ed. CBS Publishers and Distributors, New Delhi, Bangalore, 2004, 84-85.
6. Kanikkannan N, Kandimalla K, Lamba SS, Singh M. Structure-activity relationship of chemical penetration enhancers in Transdermal drug delivery. Current Medicinal Chemistry. 1999; 6(7):593-608.
7. Kataria S, Middha A, Sandhu P, Bilandi A, Kapoor B. Microsphere: a review, international journal of research in pharmacy and chemistry. 2011; 1(4):1184-1198.
8. Khan S, Tiwari T, Rao N, Joshi A, Dubey BK. A review: Microspheres, world journal of pharmacy and pharmaceutical sciences. 2012; 1(1):125-145. Lachman L, Lieberman HA., Pharmaceutical Dosage Forms, microspheres, Marcel Dekker, Inc. New York, 2000, 12.
9. Lee JH, Park TG, Lee YB, Shin SC, Choi HK. Effect of adding non-volatile oil as a core material for the floating microspheres prepared by emulsion solvent diffusion method. J Micro encap. 2001; 18:65-75.
10. Pandya K, Prajapati G, Patel MR, Patel KR, Patel NM. A review on Microspheres, International pharmaceutica sciencia. 2012; 2(2):53-57