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Research Article

Preformulation Studies of Niosomal Gel of Prednisolone & Azithromycin for Topical Drug Delivery System

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Abstract

Azithromycin is a semi-synthetic macrolide antibiotic drug, effective against a wide variety of bacteria. It is primarily used to treat the bacterial infections associated with weaker immune system. Prednisolone is a synthetic corticosteroid, used for suppressing the immune system and inflammation. When used in combination, both the drugs are very much effective in the management of inflammatory conditions or diseases in which the immune system plays an important role. The aim is to study the preformulation parameters for niosomal gel for topical use. The objective of Preformulation study is to generic information useful to the formulator in developing stable and bioavailable dosage form. The use of Preformulation parameter maximize the chances of getting a formulation which is safe, efficacious and stable product and at the same time provide optimization of the drug product quality. Administration of conventional tablets of prednisolone has been reported to exhibit delayed release and unwanted side effects so prednisolone loaded niosomes were developed and azithromycin which tend to cause allergic reaction was incorporated into gel base provide rapid penetration through skin , improve therapeutic performance, restrict action to the target cell and improve patient compliance, hence the objective of the study was made to develop sustained release gel containing azithromycin and niosomal vesicles of prednisolone using Carbopol as a polymer which will controlled the release of drug, increasing the bioavailability of the drug and thus decreasing the dosing frequency of the drug. The Preformulation studies were carried out for identification (physical appearance, melting point, and uv spectrophotometer), solubility profile, TLC, FTIR, compatibility studies, simultaneous estimation. All the observation and results showed that the azithromycin and prednisolone serve as suitable candidate for Topical drug delivery system that may improve the bioavailability.

Key words: Niosome, Topical gel, Preformulation, Compatibility, Simultaneous estimation, sustain

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1. Introduction

Topical niosomal gel has been in use for dermatological disease since very long time. Azithromycin (Figure 1) is a semi-synthetic macrolide antibiotic drug,

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effective against a wide variety of bacteria. It is primarily used to treat the bacterial infections associated with weaker immune system. Prednisolone (Figure 2) is a synthetic corticosteroid, used for suppressing the immune system and inflammation. When used in combination, both the drugs are very much effective in the management of inflammatory conditions or diseases in which the immune system plays an important role [1]. The objective of Preformulation study is to generic information useful to the formulator in developing stable and bioavailable dosage form. The use of Preformulation parameter maximize the chances in formulation an safe, efficacious and stable product and compatible, at the same time provides simultaneous estimation studies and product quality. The Preformulation studies were carried out for identification (physical appearance, solubility studies, melting point, and uv spectrophotometer, IR spectra TLC, estimation of drugs). Niosomal gel has been explored extensively for Topical application to enhance skin penetration as well as skin retention of the drug. It provides effective and immediate release of the drugs [2]. Prednisolone loaded niosomes were used to provide sustained release and protect the drug from external environment, which further are being evaluated before incorporating into gel base [3].

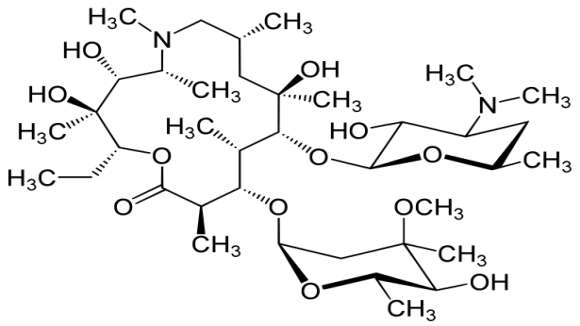


Figure 1. Chemical Structure of Azithromycin

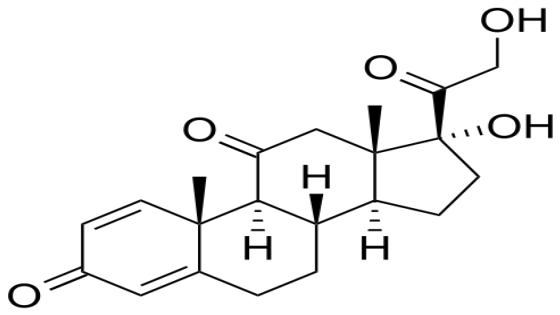


Figure 2. Chemical Structure of Prednisolone

2. Materials and Method

1. Preformulation studies of drugs
a) Azithromycin

Solubility study of azithromycin was performed in 0.1 N HCl & water. Excess of AZI was dissolved separately in the above 2 solvents and shaken continuously for 24 hours in the mechanical shaker at 25±2°C. Solutions were filtered and absorbance was recorded using UV spectrometer (Systronic 2230) and the amount of AZI dissolved in 10 ml of 0.1N HCl & water was calculated [5].

Melting point

The melting point of the drug was performed by capillary method. In this, drug was filled in the capillary tube sealed at one end to a height of 3 mm from closed end and capillary was introduced into digital melting point apparatus (SSS Pvt. Ltd.). The temperature range at which drug melt was noted down.

IR Spectroscopy

Characterization of AZI was done by FTIR spectroscopy. The drug was mixed with KBr & pressed into a very thin pellet which was then observed under IR spectrophotometer and the spectrum obtained was interpreted [6].

TLC

AZI was dissolved in acetone, applied on TLC plate & run in the saturated chamber containing mobile phase. Mobile phase was prepared by taking mixture of ethyl-acetate and hexane in the ratio 1:1(fried et al; 1999). Rf value was then calculated.

Estimation of azithromycin using UV-visible spectrophotometer

Determination of absorbance maxima (λ_{max}) of azithromycin

For the standardization of the drug by using UV spectroscopy, the drug is firstly subjected to wavelength scan for determination of absorbance maxima (λ_{max}). A stock solution (1000µg/ml) of drug was prepared by dissolving 100 mg drug in 20 ml, 0.1N HCl in 100 ml volumetric flask and volume was made up to 100 ml with respective dissolution media (phosphate buffer saline pH 6.5). The samples were scanned between range of 200-400 nm by using UV-visible spectrophotometer. The wavelength at which maximum absorbance observed was selected as the analytical wavelength of the drug for that particular buffer media.

Calibration curve of the Azithromycin

The Calibration curve of the drug was plotted in phosphate buffer saline (pH 6.5) using 0.1N HCl as a cosolvent to dissolve the drug [7]. A stock solution of drug was prepared and was serially diluted with phosphate buffer saline pH 6.5 to obtain the concentration range of 100-800µg/ml respectively. The dilutions were analyzed spectrophotometrically at λ_{max} of 298 nm using phosphate buffer saline (pH 6.5) as a blank.

b) Prednisolone

Solubility studies

Solubility study of Prednisolone was performed in methanol & water. Excess of drug was dissolved separately in the above 2 solvents and shaken continuously for 24 hours in the mechanical shaker at 25±2°C. Solutions were filtered and absorbance was recorded using UV spectrometer (Systronic 2203) and the amount of Prednisolone dissolved in 10 ml of metanol & water was calculated.

Melting point

The melting point of the drug was determined by capillary method. In this drug the was filled in the capillary tube sealed at one end to a height of 3 mm from closed end and capillary was introduced into digital melting point apparatus. The temperature range at which drug melts was noted down.

IR Spectroscopy

Characterization of Prednisolone was done by FTIR spectroscopy. The drug was mixed with KBr & pressed into a very thin pellet which was then observed under IR spectrophotometer (FTIR 8400 S, Shimadzu) and the spectrum obtained was interpreted.

TLC

Prednisolone was dissolved in acetone, applied on TLC plate & run in the saturated chamber containing mobile phase. Mobile phase was prepared by taking mixture of ethyl-acetate and hexane in the ratio 1:1(fried et al; 1999). Rf value was then calculated.

Estimation of Prednisolone using UV-visible spectrophotometer

Determination of absorbance maxima (λ_{max}) of Prednisolone

For the standardization of the drug using UV spectroscopy, the drug is firstly subjected to wavelength scan for

determination of absorbance maxima (λ_{max}) [8]. A stock solution (1000µg/ml) of drug was prepared by dissolving 100 mg drug in 20 ml of methanol in 100 ml volumetric flask and volume was made up to 100 ml with respective dissolution media (phosphate buffer saline pH 6.5). The samples were scanned over the range of 200-400 nm by using UV-visible spectrophotometer. The wavelength at which maximum absorbance observed was selected as the analytical wavelength of the drug for that particular buffer media.

Calibration curve of prednisolone

The Calibration curve of the drug was plotted in phosphate buffer saline pH 6.5 using methanol as a cosolvent to dissolve the drug. A stock solution of drug was prepared and was serially diluted with phosphate buffer saline pH 6.5 to obtain the concentration of 10-50µg/ml respectively. The dilutions were analyzed spectrophotometrically at λ_{max} of 245 nm using methanol and phosphate buffer saline pH 6.5 as a blank [9].

Compatibility studies

The specified amount of drugs and the excipients (Span 60, cholesterol, carbopol, triethanolamine, methyl paraben, propylene glycol, glycerin) were weighed separately, mixed in ratio of 1:1 and filled in seprate vials. The vials were then stored under 2 different conditions at 2-8°C & 40° C with 75% RH. Observations of all mixture were done on 0^{th} day, 15^{th}day & 30^{th} day. The compatibility of drugs with oily bases (Span 60, cholesterol, carbopol, triethanolamine, methyl paraben, propylene glycol, glycerin) was studied by thin layer chromatography [10].

Simultaneous estimation of Azithromycin & Prednisolone

Preparation of standard stock solution

Accurately weighed 10 mg of AZI was transferred to 10 ml volumetric flask and volume was made up with 0.1 N HCl (used as cosolvent as AZI is not completely soluble in phosphate buffer saline (pH 6.5) to get a solution of concentration 1000µg/ml. 1 ml of stock solution was diluted with phosphate buffer saline (pH 6.5) upto 10 ml to get a concentration of 100µg/ml and then further dilutions were made to obtain the concentration range of 10-45µg/ml using phosphate buffer saline (pH 6.5). Solution of PRE was prepared in methanol (used as cosolvent) in a similar way to obtain the concentration range of 10-90µg/ml by dilution with phosphate buffer saline (pH 6.5). Both the solutions were scanned in the spectrum mode over the range 200-400nm [11].

Concentration of each component was determined by using simultaneous estimation equation

$A_1 = E_{1a} C_1 + E_{2a} C_2$ ..... 298 nm

$A_2 = E_{1b} C_1 + E_{2b} C_2$ ..... 245 nm

A_1 =absorbance value of sample at 298 nm
A_2 =absorbance value of sample at 245 nm
E_{1a} = absopitivity of 1^{st} drug at 298 nm
E_{1b} = absopitivity of 1^{st} drug at 245 nm
E_{2a} = absopitivity of 2^{nd} drug at 298 nm
E_{2b} = absopitivity of 2^{nd} drug at 245 nm
C_1 = concentration of 1^{st} drug in µg/ml
C_2 = concentration of 2^{nd} drug in µg/ml

The absorbance of sample solutions of AZI and PRE were measured at 298 nm and 245 nm respectively. The results were calculated by the following formula using Vierodt's method. [15]

A1 = ax1 Cx + ax2 Cy at 298 nm
A2 = ay1 Cx + ay2 Cy at 245 nm

Where,
A1 and A2 are absorbance of diluted mixture of drugs at 298 nm and 245 nm

respectively, Cx and Cy are the concentration of AZI and PRE respectively (µg/ml), ax1 and ax2 are absorptivities of AZI at 298 nm and 245 nm respectively, ay1 and ay2 are absorptivities of PRE at 298 nm and 245 nm respectively [12].

3. Result and Discussion

Solubility Study

Azithromycin was found to be Insoluble in water, ethanol, phosphate buffer saline, sparingly soluble in methanol, soluble in 0.1N HCl (Table 1). Prednisolone was very slightly soluble in water, soluble in methanol, dioxane. (Table 2).

Table 1. Solubility study of Azithromycin

Solvent	Solubility (mg/ml)
Water	0.0076
0.1N HCl	29.23

Table 2. Solubility study of Prednisolone

Solvent	Solubility (mg/ml)
Water	0.0081
Methanol	39.23

Melting point

Observed melting point (Table 3) of azithromycin and prednisolone was found similar to reported one which shows drugs are pure and stable.

Table 3. Melting point of Azithromycin & Prednisolone

Drug	Experimental	Observe/ Reported
Azithromycin	114^{0}C	113^{0}C
Prednisolone	243 ^{0}C	240 ^{0}C

IR Interpretation

IR spectra of azithromycin and prednisolone was obtained and interpreted by identifying the the value of characteristics (Figure 3 & Figure 4) showed sharp peak at 1650-1850 cm^{-1} corresponding to stretching vibration of carbonyl group. Identification and purity of prednisolone were interpreted three characteristic bands corresponding to carbonyl group at 1622cm^{-1}(α-β unsaturated groups), 1688cm^{-1} (aliphatic group), 1707 cm^{-1}(cyclic group)

Thin Layer Chromatography

The Rf value of azithromycin wwas found to be 0.867 in ethyl acetate :hexane (1:1) and Rf value of prednisolone was found to be 0.934.

Estimation of azithromycin and prednisolone

Determination of absorbance maxima (λ_{max}) of azithromycin and prednisolone

The stock solution of the drug was scanned in the wavelength range of 200-400 nm using UV spectrophotometer. The absorption maximum (λ_{max}) of azithromycin and prednisolone was found to be 298 nm (Figure 5) and 245 nm (Figure 6).

Standard plot of azithromycin in phosphate buffer saline (pH 6.5)

Calibration curve (Figure 7) of different concentration of drug in phosphate buffer saline (pH 6.5) metanol vs. absorbance was found to linear and beer's lay obeyed in the concentration range of 100-800µg/ml (R^2=0.992) (Table 4).

Table 4. Concentration v/s Absorbance

Concenteration (µg/ml)	**Absorbance**
0	0
100	0.014
200	0.025
300	0.038
400	0.051
500	0.062
600	0.075
700	0.091
800	0.106

Standard plot of Prednisolone in phosphate buffer saline (pH 6.5)

Calibration curve (Figure 8) of different concentration of drug in phosphate buffer saline (pH 6.5) vs. absorbance was found to linear and beer's lay obeyed in the concentration range of 10-80µg/ml (R^2=0.999) (Table 5).

Table 5. Concentration v/s Absorbance

Concenteration (µg/ml)	Absorbance(nm)
0	0
10	0.103
20	0.210
30	0.301
40	0.398
50	0.501

Compatibility studies

From drug:excipients compatibility studies the results depicts there was no change in color, no lump formation occurred in any of the mixture at different temperature & humidity conditions, when observed on different days (7^{th}, 15, 30^{th} days) interval in comparison to initial observation on 0^{th} day. This confirmed that both the drugs were compatible with each other as well as with excipients. (Figure 9). R_f values obtained from TLC studies on (7^{th}, 15, 30^{th} days) were approximately similar to R_f values of pure drugs and gel excipients obtained on 0^{th} day (Figure 10), predicting the compatibility of both drugs with gel excipients.

Simultaneous estimation

The drugs mixture were scanned over the range of 200-400nm by using UV-visible spectrophotometer. λ_{max} for azithromycin and prednisolone was found to be 298nm & 245nm as shown in (figure 11). Overlaid spectra of azithromycin and prednisolone (figure 12).

Estimation of C_1& C_2 for azithromycin & prednisolone

Table 5. Various parameters and concentration of drug calculated for simultaneous of drugs by veirodicts method.

A1	0.262			
A2	0.54	Drug obtained	Concentration taken	Concentration
E1a	0.124		µg/ml	µg/ml
E1b	0.172	Azithromycin	10	9.818
E2a	0.062	Prednisolone	10	9.101
E2b	0.0006			

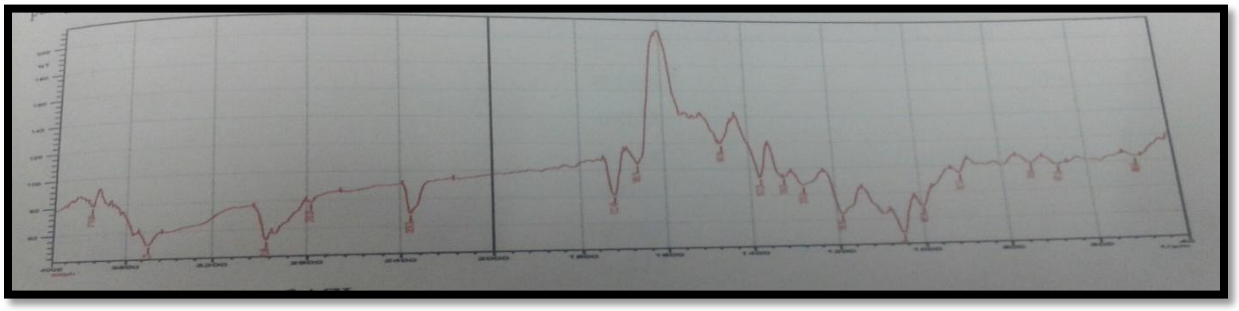


Figure 3. IR spectra of azithromycin

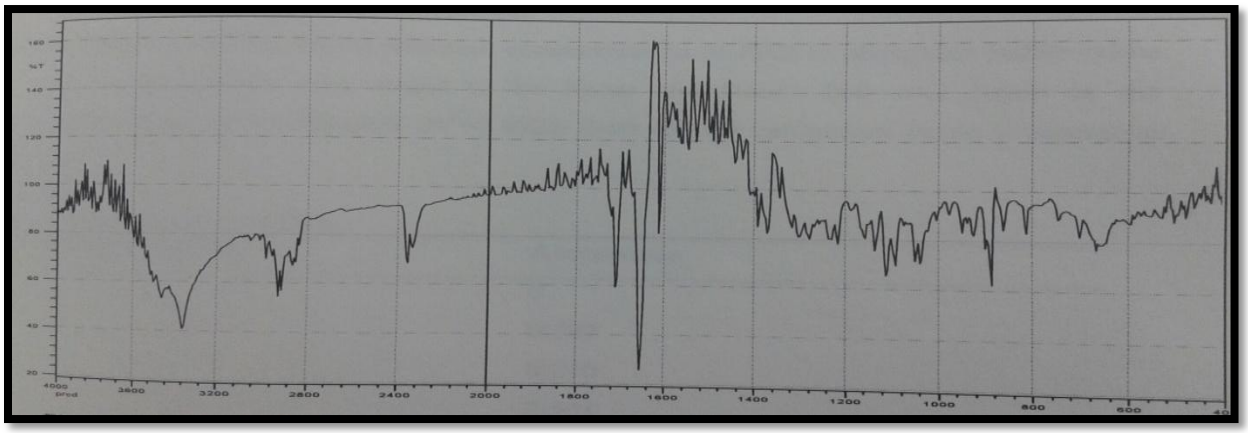


Figure 4. IR spectra of prednisolone

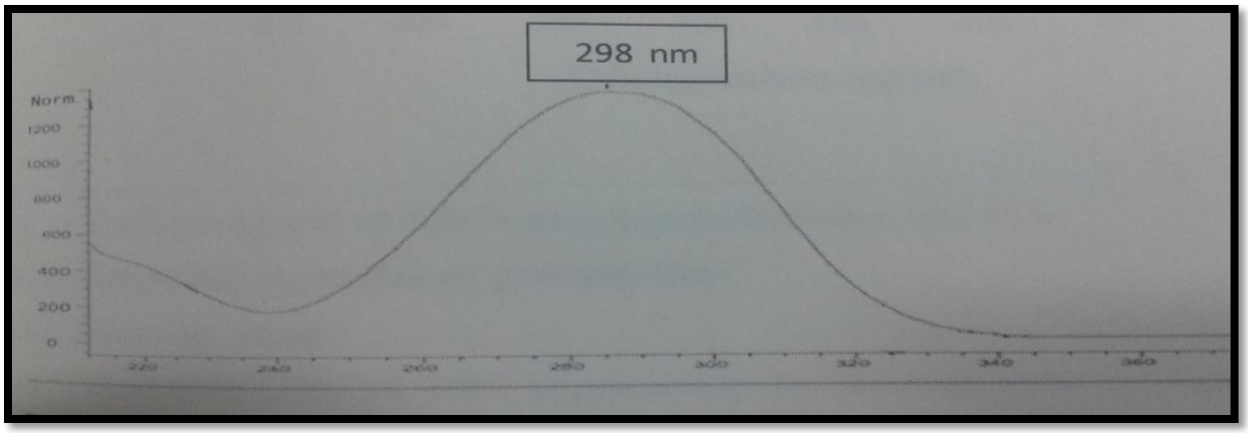


Figure 5. Absorbance maxima (λ_{max}) of azithromycin

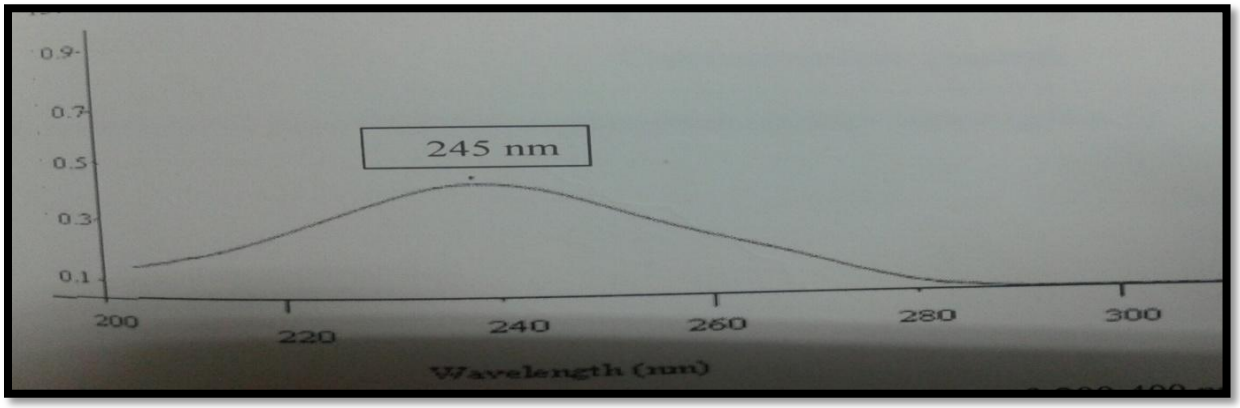


Figure 6. Absorbance maxima (λ_{max}) of prednisolone

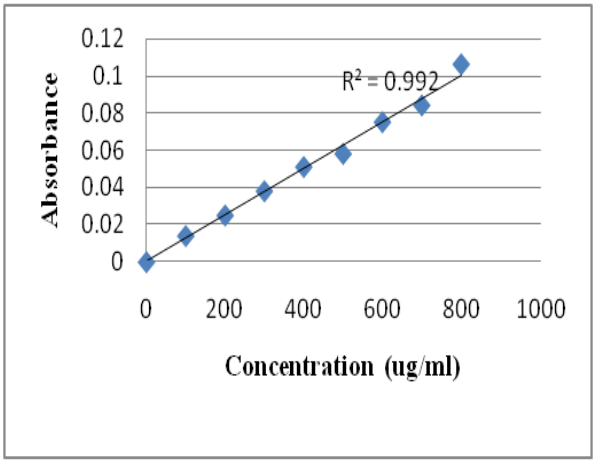


Figure 7. Calibration curve of Azithromycin

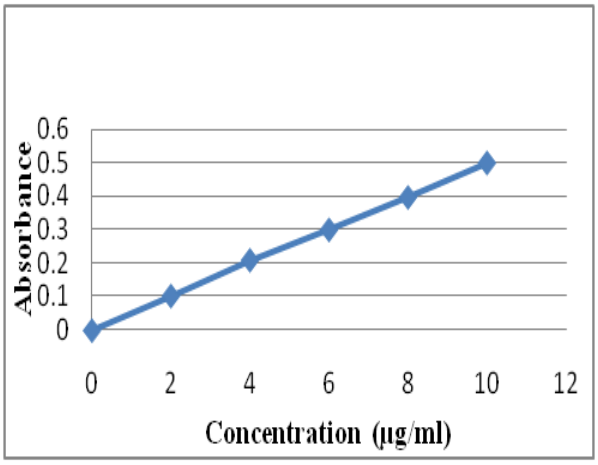
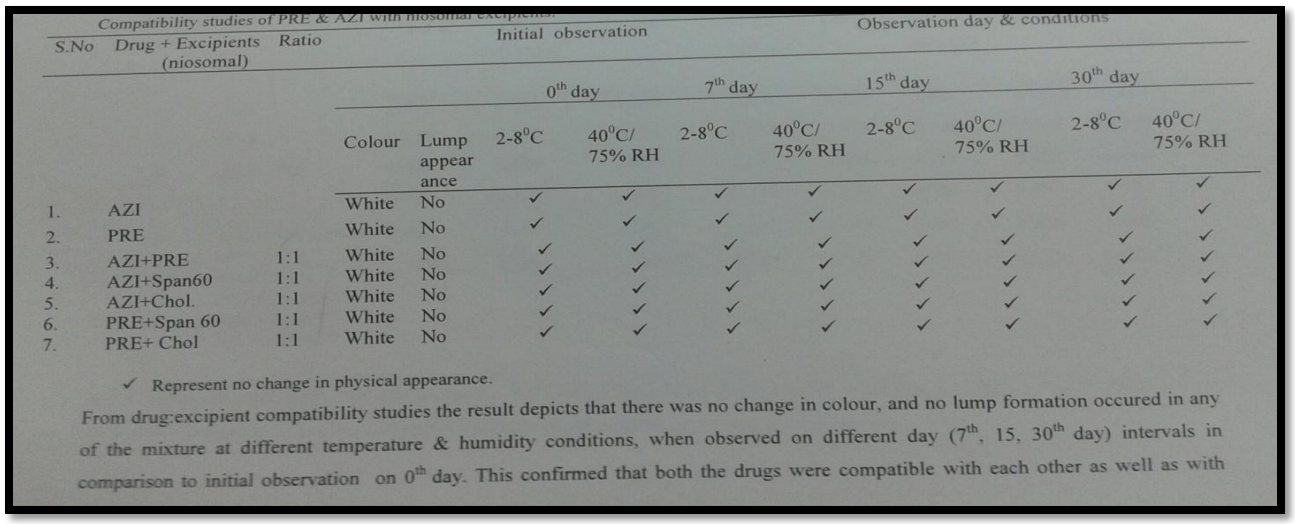


Figure 8. Calibration curve of prednisolone



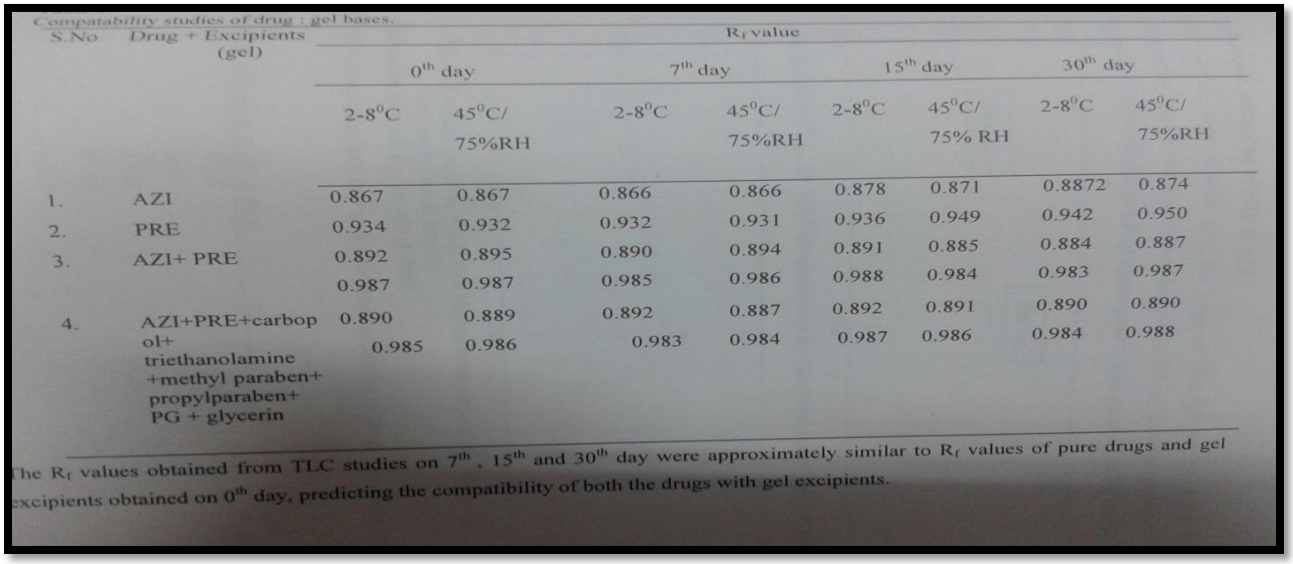
Compatibility studies of PRE & AZI with niosomal excipients.

S.No	Drug + Excipients (niosomal)	Ratio	Initial observation		Observation day & conditions					
					0th day		7th day		15th day		30th day	
			Colour	Lump appearance	2-8°C	40°C/ 75% RH	2-8°C	40°C/ 75% RH	2-8°C	40°C/ 75% RH	2-8°C	40°C/ 75% RH
1.	AZI		White	No	✓	✓	✓	✓	✓	✓	✓	✓
2.	PRE		White	No	✓	✓	✓	✓	✓	✓	✓	✓
3.	AZI+PRE	1:1	White	No	✓	✓	✓	✓	✓	✓	✓	✓
4.	AZI+Span60	1:1	White	No	✓	✓	✓	✓	✓	✓	✓	✓
5.	AZI+Chol.	1:1	White	No	✓	✓	✓	✓	✓	✓	✓	✓
6.	PRE+Span 60	1:1	White	No	✓	✓	✓	✓	✓	✓	✓	✓
7.	PRE+ Chol	1:1	White	No	✓	✓	✓	✓	✓	✓	✓	✓

✓ Represent no change in physical appearance.

From drug:excipient compatibility studies the result depicts that there was no change in colour, and no lump formation occured in any of the mixture at different temperature & humidity conditions, when observed on different day (7th, 15, 30th day) intervals in comparison to initial observation on 0th day. This confirmed that both the drugs were compatible with each other as well as with

Figure 9. Compatibility study of azithromycin and prednisolone with excipients



Compatability studies of drug : gel bases.

S.No	Drug + Excipients (gel)	R_f value							
		0th day		7th day		15th day		30th day	
		2-8°C	45°C/ 75%RH	2-8°C	45°C/ 75%RH	2-8°C	45°C/ 75% RH	2-8°C	45°C/ 75%RH
1.	AZI	0.867	0.867	0.866	0.866	0.878	0.871	0.8872	0.874
2.	PRE	0.934	0.932	0.932	0.931	0.936	0.949	0.942	0.950
3.	AZI+ PRE	0.892	0.895	0.890	0.894	0.891	0.885	0.884	0.887
		0.987	0.987	0.985	0.986	0.988	0.984	0.983	0.987
4.	AZI+PRE+carbopol+ triethanolamine +methyl paraben+ propylparaben+ PG + glycerin	0.890	0.889	0.892	0.887	0.892	0.891	0.890	0.890
		0.985	0.986	0.983	0.984	0.987	0.986	0.984	0.988

The R_f values obtained from TLC studies on 7th , 15th and 30th day were approximately similar to R_f values of pure drugs and gel excipients obtained on 0th day, predicting the compatibility of both the drugs with gel excipients.

Figure 10. R_f studies of compatibility studies

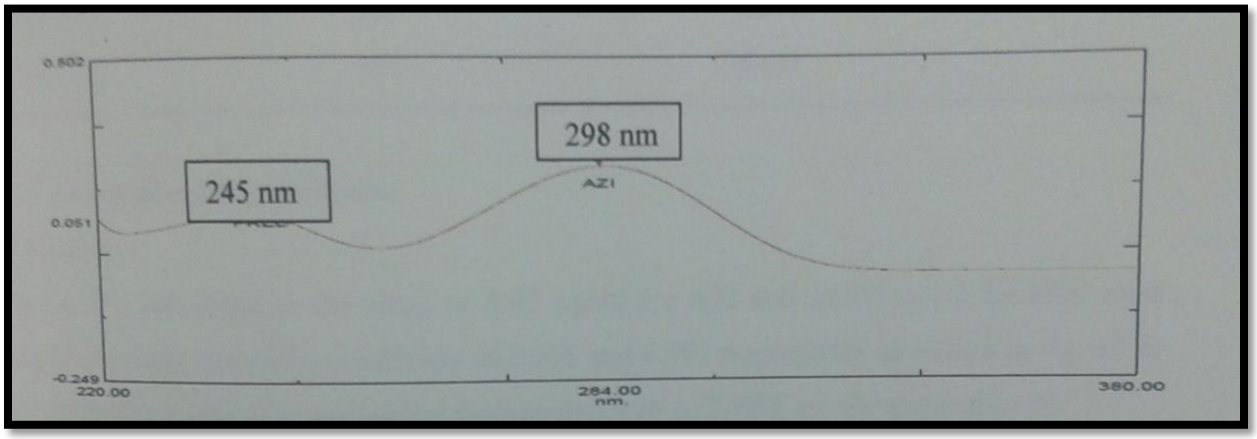


Figure 11. λ_{max} for azithromycin and prednisolone

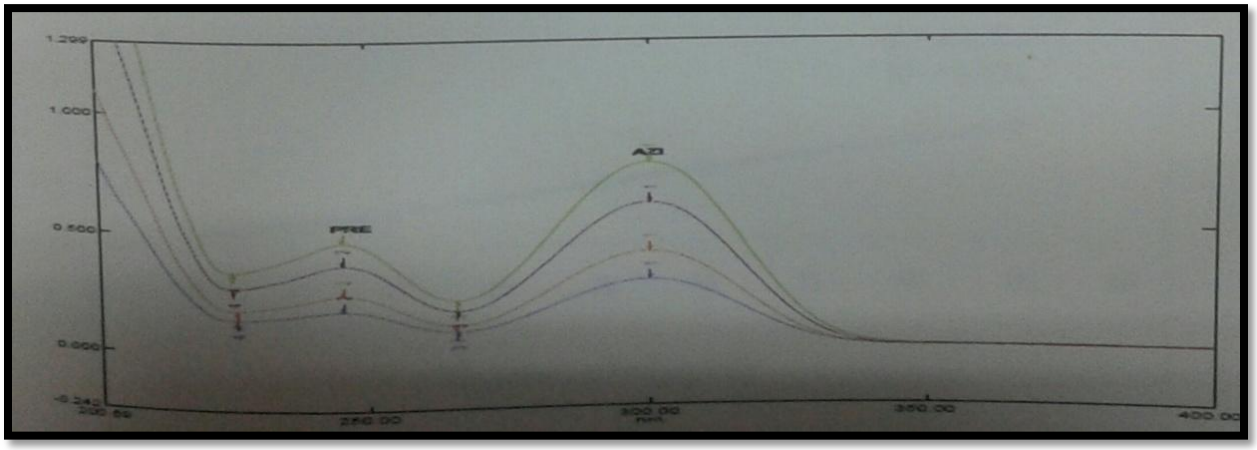


Figure 12. λ_{max} for azithromycin and prednisolone

Conclusion

Preformulation study gives brief idea about the identification (physical appearance, solubility studies, melting point, and uv spectrophotometer, IR spectra TLC, estimation of drugs). On the basis of this study we conclude that the combinations of both drugs (azithromycin and predinsolone) can be use in the management of skin diseases like psoriasis.

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