CHAPTER III

MATERIALS AND METHODS

Materials

1. Nifedipine powder, USP XXI (Suppl 7), potency 99.10% (Wilhelm Weizien & Co.), Batch No. 313
2. Pluronic F-127 (BASF), Lot No. 9864759
3. Sodium bisulfite, analytical grade (Mallinckrodt Inc.), Lot No. 7448 KCLZ
4. Absolute ethanol, AR. (E. Merck), Lot No. 045 K14927283

Apparatus

1. pH meter (Model SA 520, Orion Research Inc., USA.)
2. Analytical balance (Sartorius 1615 MP, Germany)
3. Spectrophotometer (Spectronic 2000, Bausch & Lomb, USA.)
4. Fluorescent lamp, daylight, 15 watt, 43 cm long (Toshiba, Japan)
5. Light cabinet

Method

A. Preparation of 1% w/w Nifedipine in Pluronic F-127 Gel

1. Formulation

Every formulation contained the fixed concentration of nifedipine (1% w/w) which was dispersed in 40% w/w Pluronic F-127 gel. Sodium bisulfite was added to nifedipine gel with certain concentrations in order to study the effect of the antioxidant. There were seven formulations:-

formulation Ia : 1% w/w of nifedipine in Pluronic F-127 gel,under accelerated light,

formulation Ib : 1% w/w of nifedipine in Pluronic F-127 gel, in normal light,

formulation Ic : 1% w/w of nifedipine in Pluronic F-127 gel wrapped in aluminium foil, under accelerated light,

formulation II : 1% w/w of nifedipine in Pluronic F-127 gel containing sodium bisulfite 0.05% w/w, under accelerated light,

formulation III: 1% w/w of nifedipine in Pluronic F-127 gel containing sodium bisulfite 0.10% w/w, under accelerated light,

formulation IV : 1% w/w of nifedipine in Pluronic F-127 gel containing sodium bisulfite 0.30% w/w, under accelerated light,

formulation V : 1% w/w of nifedipine in Pluronic F-127 gel containing sodium bisulfite 0.50% w/w, under accelerated light.

2. Preparation of Pluronic F-127 gel

The Pluronic F-127 gel was prepared by cold method (Gilbert, Richardson et al., 1987; Miyazaki et al., 1986). From preliminary study, the optimum concentration of Pluronic F-127 gel in water to obtain stable gel was 40% w/w. A weighed amount of Pluronic F-127 was slowly added to cold water (about $5^{o}C$) at concentration of 40% w/w with gentle mixing. This dispersion was then placed in a refrigerator to ensure

complete dissolution. Eventually, a clear and viscous solution was formed and transformed to gel state when the solution was later stored at room temperature.

For the formulations containing sodium bisulfite, gel bases were prepared by a slightly modified method in order to optimise mixing. Sodium bisulfite (at required amounts) had been dissolved in cold water prior to Pluronic F-127 addition.

3. Preparation of nifedipine in Pluronic F-127 gel

An accurately weighed amount of nifedipine was triturated with 40% w/w Pluronic F-127 gel, with or without sodium bisulfite, using geometric dilution technique, until homogeneous gel was formed. Then the approximately equal parts of this preparation were filled into the bottom of 5-ml clear vials and fixed the thickness of this preparation to be not more than 0.3 cm. The vials were tightly sealed and protected from light prior to further study.

Additionally, it should be emphasized that all experimental processes were carried on by strictly kept from light. The studies were performed in dark room and all of glasswares used were priorly wrapped in aluminium foil.

B. Analysis of Nifedipine in Pluronic F-127 Gel

The UV spectrophotometric method using the multi-component analysis was used for the determination of concentrations of reduced form of nifedipine in gels before and after irradiation according to Al-Turk et al. (1989).

1. Determination of the maxima absorption wavelength (λmax) of reduced and oxidized forms.

A 50-ml solution of nifedipine with concentration of 3.403×10^{-5} M was prepared in the solution mixture of 95% ethanol and 0.198 g of 40% w/w Pluronic F-127 gel, the medium of which was corresponded to that of formulation I. Immediately, the maximum absorption wavelength of reduced form of nifedipine was determined by UV spectrophotometer (Appendix B). Additionally, in order to ensure that there was no interference occurred from Pluronic F-127 gel, the UV absorption spectrum of the gel was also studied.

To determine the λ_{max} of the oxidized form, the solution of nifedipine was irradiated under fluorescent light (15 watt) placed 30 cm above the solution in the light cabinet for 4 hours and then the λ_{max} of irradiated solution was determined (Appendix B).

To determine the λ_{max} of nifedipine in the presence of sodium bisulfite, the 50-ml solutions of nifedipine with concentration of 3.403×10^{-5} M were prepared in the solution mixtures of 95% ethanol and 0.198 g of 40% w/w Pluronic F-127 gel containing specified amount of sodium bisulfite, the media of which were corresponded to those of formulations II-V. Then, the λ_{max} of the spectra of the reduced form of nifedipine were immediately determined by UV spectrophotometer. Similarly, to ensure that there was no interference occurred from sodium bisulfite, the absorption spectrum of the sodium bisulfite solution was studied.

To determine the λ_{max} of the oxidized form of nifedipine in the presence of sodium bisulfite, these solutions were irradiated in the similar method as described above. And then, the λ_{max} of the solutions were determined.

2. Determination of the molar absorptivities

The 50-ml solutions of nifedipine with concentrations of 1.701×10^{-5} - 14.178×10^{-5} M were prepared in the solution mixtures of 95% ethanol and 0.198 g of 40% w/w Pluronic F-127 gel, the media of which were corresponded to that of formulation I. Immediately,

the absorbances at 281 and 334 nm, the λ_{max} of oxidized and reduced form obtained from the previous study, were determined. Absorbances versus molar concentrations were fitted to a straight line using linear regression (Appendix B).

These solutions were irradiated under fluorescent light (15 watt) placed 30 cm above the solutions for 4 hours and then absorbances were measured at 281 and 334 nm. Absorbances versus molar concentrations were fitted to a straight line using linear regression (Appendix B). To ensure that the oxidation of nifedipine was completed, irradiation was prolonged to 7, 24 and 48 hours, and the absorbances were measured at the end of each irradiation. The slopes (as molar absorptivities) of the curves at 7, 24, 48 hours were compared with that at 4 hours by utilizing Student's t-test.

For the solutions of nifedipine containing sodium bisulfite, the media of which were corresponded to those of the formulations II-V, the procedure for determining the molar absorptivities of the reduced and oxidized forms was similar to that for the solutions without sodium bisulfite (Appendix B).

From plots of absorbances versus molar concentrations, the slopes (as molar absorptivities) and

y-intercepts of the straight lines were obtained, and used for establishment the equations for calculating the concentrations of the reduced form in the solutions (Appendix B) (modified from Al-Turk et al., 1989).

3. Analysis of nifedipine in Pluronic F-127 gel

An accurately weighed 0.2000 g of nifedipine gel was dissolved and made to volume of 50 ml in 95% ethanol. Absorbances at 281 and 334 nm were determined and then the concentration of reduced form was calculated.

C. Photostability Study of Nifedipine Gel

Physical stability study

Physical appearances including color and pH of the gel preparations were studied before irradiation. In the physical stability study, the assessment of color of the gels was used before and after irradiation.

Chemical stability study

Before and after irradiation, all preparations were determined for their % labeled amount and % remaining of nifedipine, respectively, using the analytical method as in B-3.

For all formulations, except Ib, the photostability studies were performed under accelerated light. The vials containing these gel preparations were irradiated under a fluorescent lamp (15 watt, 43 cm long) placed 30 cm away from the samples in the light cabinet, modified from the light cabinet of Lachman and Cooper (1959). The temperature was 26 ± 3°C.

For formulation Ib, the study was performed under normal light. The vials containing the gel preparation were irradiated under normal laboratory light (a mixture of fluorescent light and diffuse sunlight). Irradiation was limited at only 10-12 hours per day in daytime. The temperature was 26 ± 3°C.

All preparations were irradiated for 116 days. For all formulations, except formulation Ib, samples were collected by removing three vials of samples after 0.5, 1, 2, 4, 8 hours, 1, 1.25, 2, 3, 4,.....86, 116 days of exposure. For formulation Ib, samples were collected after 1, 2, 3,..., 86, 116 days of exposure.

D. Evaluation of the Photostability Studies

Physical stability study

The color change of the gel preparations after irradiation was observed and then recorded.

Chemical stability study

(a) To determine the reaction kinetics and order of reaction.

Plots of % remaining of nifedipine versus time, ln % remaining of nifedipine versus time and (% remaining)$^{-1}$ of nifedipine versus time were studied, using linear regression to determine slopes and coefficients of determination (r^2),

zero order reaction,

$$C = C_o - kt \qquad \text{(Eq. 10)},$$

first order reaction,

$$\ln C = \ln C_o - kt \qquad \text{(Eq. 11)},$$

second order reaction,

$$\frac{1}{C} = \frac{1}{C_o} + kt \qquad \text{(Eq. 12)},$$

where C_o= the initial concentration of drug,

C = the concentration of drug at time t,

k = the degradation rate constant

The order of reaction would be obtained from the plot which was a good fit to a straight line (r^2 was nearest to 1).

(b) To determine the degradation rate constants

zero order reaction,

k = (-slope) of the plot following (Eq 10),

first order reaction,

k = (-slope) of the plot following (Eq 11),

second order reaction,

k = slope of the plot following (Eq 12).

The degradation rate constants of different formulations were compared using Student's t-test, One Way Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test at the significant level of $\alpha = 0.05$.

(c) To determine the shelf-lives

Shelf-life was the time required for the drug concentration was 90% of its original value, i.e., 10% degradation. The shelf-lives were predicted as follows :

zero order reaction,

$$t_{0.9} = \frac{C_o}{10k} \qquad \text{(Eq 13)},$$

first order reaction,

$$t_{0.9} = \frac{0.105}{k} \qquad \text{(Eq 14)},$$

second order reaction,

$$t_{0.9} = \frac{0.11}{C_o k} \qquad \text{(Eq. 15)},$$

where $t_{0.9}$ = shelf-life.

The shelf-lives of different formulations were compared using Student's test, One Way Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test at the significant level of $\alpha = 0.05$.

(d) To determine the effect of concentration of sodium bisulfite on photostability of nifedipine gel.

The plot of degradation rate constants versus concentrations of sodium bisulfite was studied. The correlation coefficient (r) was determined using linear regression. The relationship between the concentrations of sodium bisulfite and the degradation rate constants of nifedipine was then analyzed by the pearson's correlation coefficient test. The level of significant was 10% because the sample size was small.