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ANALYTICAL STUDY OF NIFEDIPINE AND ITS PHOTO - OXIDIZED FORM

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ABSTRACT

Nifedipine, a calcium channel blocking dihydropyridine derivative, undergoes ready photochemical conversion to the corresponding pyridine derivative when exposed to normal laboratory and UV light. The Photo-Oxidation of the drug leads to remarkable diminution of pharmacological activity.

The study describes a direct and simple spectrophotometric method for the simultaneous determination of both nifedipine and its oxidized degradation product. The molar absorptivities of both components at 237 , 280 and 360 nm were also calculated. The method is based on the measurement of absorbance values at two wavelengths and the calculation of the concentrations of the two components in the mixture by solving for two simultaneous equations.

INTRODUCTION

Nifedipine is a compound classified pharmacologically as a calcium channel blocking agent and causes relaxation of vascular smooth muscle (1).Chemically, it is a 4-(2-nitrophenyl)-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine. It is photo-sensitive and undergoes photochemical conversion to 4-(2-nitrosophenyl)-2,6-dimethyl-3,5-dicarbomethoxy pyridine when exposed to normal laboratory and UV light (2,3). The photo-oxidation of nifedipine to the pyridine derivative leads to a remarkable diminution of the pharmacological activity (4,5).

The determination of nifedipine and its oxidized degradation products has been the subject of many investigations. A high performance liquid chromatography procedure (6) for identification and separation of nifedipine and its metabolites in the biological fluids, has been employed for the analysis of the photo-oxidation products of the drug,nitrophenyl pyridine and nitrosophenyl pyridine. Also, Jakobsen et al.(7), designed a gas charomatographic condition to determine nifedipine and one of its oxidized metabolites in plasma. Such analytical procedures are superior to the indirect method that involvs oxidation of nifedipine to the more stable nitropyridine derivative prior to analysis (8,9). Although, Boje et al. (10) incorporated hydroquinone as an antioxidant during chromatographic analysis in order to protect against the oxidation of the drug on the column, Dokladalova et al. (11) in their work have shown that nifedipine does not oxidize significantly to nitropyridine during analysis.

In this study, a direct and simple spectrophotometric method for simultaneous determination of both nifedipine and its oxidized degradation product was used despite their overlapping UV absorption spectra.

MATERIALS AND METHODS

A stock solution of nifedipine (1×10^{-2} M) (pfizer Co. NY, USA) was prepared in 95% ethyl alcohol, transfered to a brown bottle, wrapped with aluminum foil, and stored in the dark at room temperature. This solution was suitably diluted in order to give absorbance values of 0.2-0.8 units. All transfers and dilutions were carried out in a darkened room. Preliminary studies to determine the spectra of the compound were performed by scanning in a wavelength range of 200-700 nm. Absorption maxima were found at 360, and 237 nm at zero time. The same solution was irradiated under fluorescent light. The spectra of the irradiated solution showed a decrease in the absorption maxima at 237 and 360 nm and the appearance of a new absorption maximum at 280 nm. This served as an indication that the compound underwent photo-oxidation.

Determination of The Molar Absorptivities

Since the absorption spectra differ for the reduced and the oxidized forms of nifedipine, multi-component analysis could be employed to determine the concentration of both molecular species. For each wavelength of maximum absorption, the total absorbance is assumed to be due to an additive effect of the two species in solution:

$$A_{237} = E^{R}_{237}\, D\, C_R + E^{O}_{237} D\, C_O \qquad (1)$$

$$A_{280} = E^{R}_{280}\, D\, C_R + E^{O}_{280} D\, C_O \qquad (2)$$

$$A_{360} = E^{R}_{360}\, D\, C_R + E^{O}_{360} D\, C_O \qquad (3)$$

Where:

A = The total absorbance values of all the molecular species at a given wavelength.

E = Molar absorptivity of the oxidized or reduced forms at a given wavelength.

D = Path length = 1 cm

C_R = Molar concentration of the reduced form

C_O = Molar concentration of the oxidized form

Solving the simutaneous equations (1) and (2) using the relationships for 237 and 280 nm, the following equations are obtained :

$$C_R = \frac{E^{O}_{280}\, A_{237} - E^{O}_{237}\, A_{280}}{E^{R}_{237}\, E^{O}_{280} - E^{O}_{237}\, E^{R}_{280}} \qquad (4)$$

$$C_O = \frac{E^{R}_{237}\, A_{280} - E^{R}_{280}\, A_{237}}{E^{R}_{237}\, E^{O}_{280} - E^{O}_{237}\, E^{R}_{280}} \qquad (5)$$

RESULTS AND DISCUSSION

Berson and Brown (12), studied the photo-oxidation of 2,6-dimethyl-3,5-dicarboethoxy-4-(2-nitrophenyl)-1,4-dihydropyridine with different ester or ketone groups at the 3,5-positions. The photo-oxidation product showed basic proper-

ties due to the pyridine nucleus. In addition, the authors attributed the appearance of the absorption maximum at 280 nm corresponding to that of nitrosopyridine, which resulted from photo-oxidation and loss of one molecule of water from the dihydropyridine molecule.

In this investigation photo-oxidation of nifedipine under fluorescent light was accomplished under conditions similar to that of Berson and Brown (12). The spectrum of 2×10^{-4}M in 95% ethanol (Figure 1) showed absorption maxima at 237 and 360 nm before irradiation. This pre-irradiated solution and its corresponding spectra are referred to as the reduced form. In the photo-chemical conversion of the reduced form, proved to be the dihydropyridine, the absorption spectra after 4 hours of irradiation showed a decrease in the absorption maixma at 237 and 360 nm and the appearance of a new absorption maximum at 280 nm (Figure 1). This post-irradiated product and its corresponding spectrum are referred to as the oxidized form and is believed to correspond to the nitrosopyridine product of Berson and Brown (12).

The analysis of both nifedipine and its oxidized degradation product in this investigation is based on the measurement of absorbance values at two wavelengths and the subsequent calculation of the concentrations of the two components in the mixture by solving for two simultaneous equations.

A series of nifedipine concentrations (10^{-5} - 10^{-4}M) were irradiated under the fluorescent light placed 30 cm

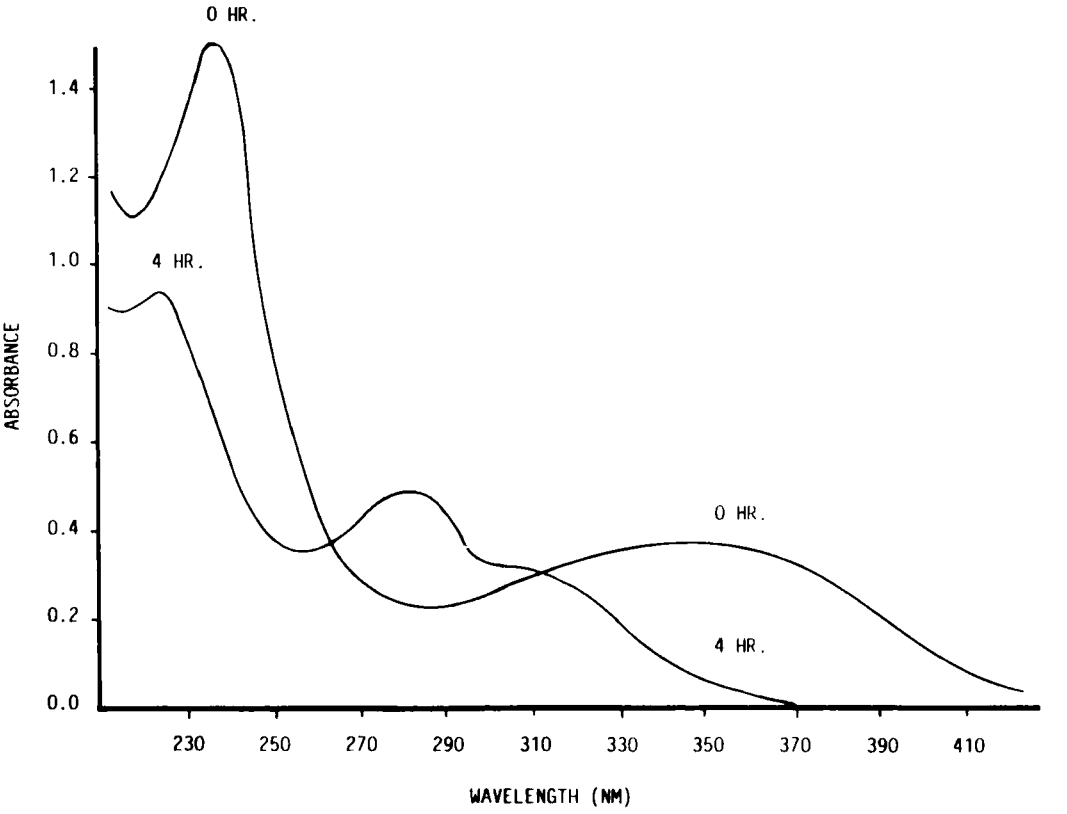

FIGURE 1

Spectral Changes of Nifedipine (2×10^{-4} M) in 95% Ethanol on Photo-Oxidation.

above the solutions in a darkened room. Beer's law plots of the absorbance values at 237 and 280 nm versus molar concentrations before irradiation were obtained as shown in Figures 2 and 3, respectively, while Figures 4 and 5 show the Beer's plots obtained after four hours of irradiation of the same solutions. The absorbance values at 280 nm obtained after irradiation correspond to the complete formation of

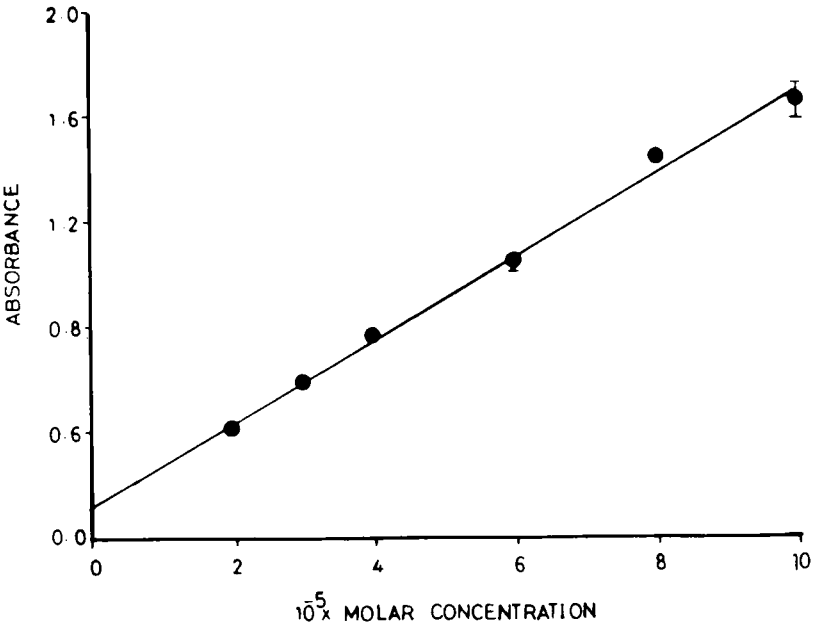

FIGURE 2

Plot of Absorbance of Nifedipine at 237 nm VS. Molar Concentration.

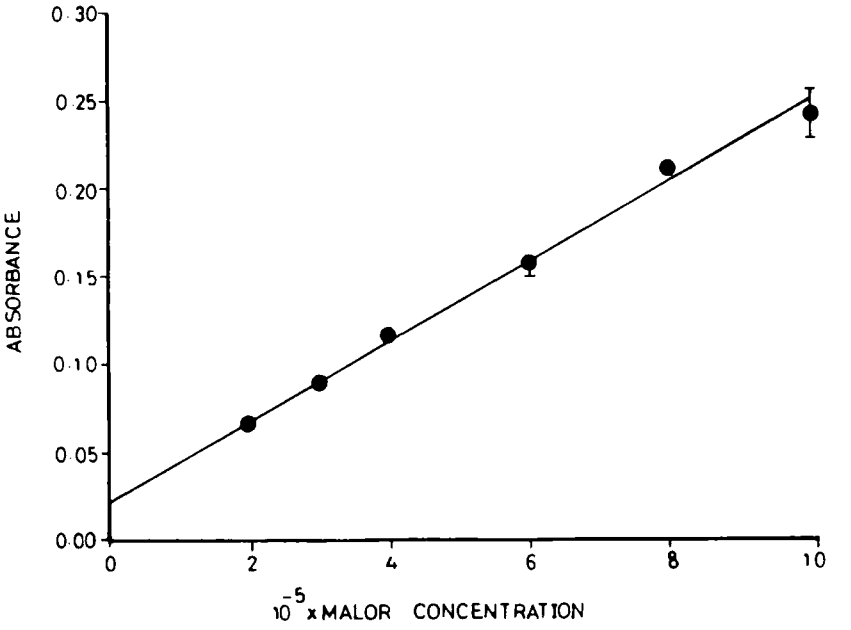

FIGURE 3

Plot of Absorbance of Nifedipine at 280 nm VS. Molar Concentration.

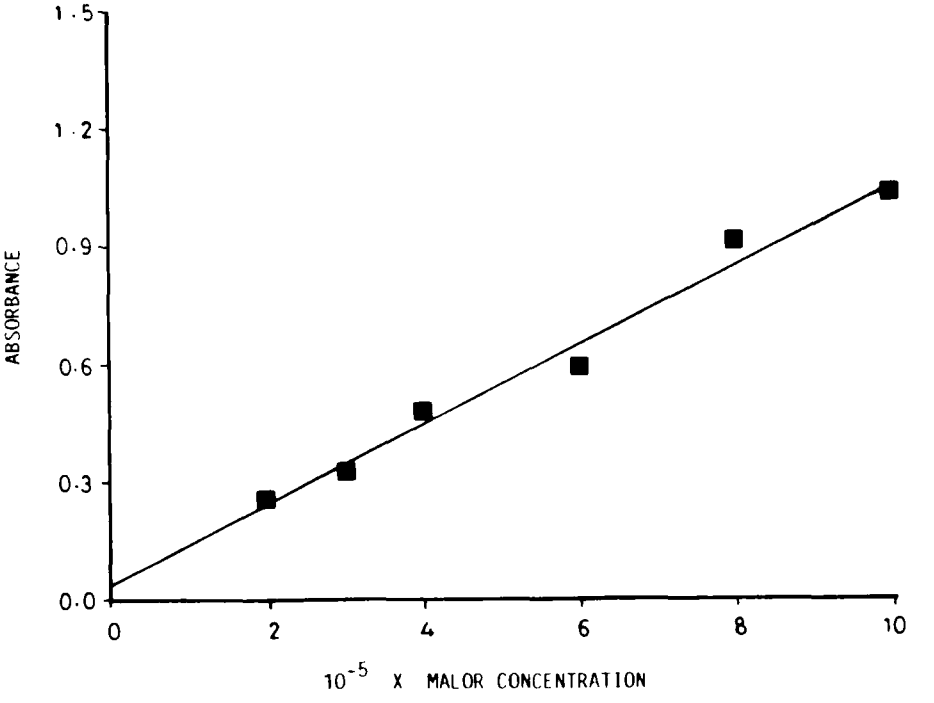

FIGURE4

Plot of Absorbance of the Oxidized from of Nifedipine at 237 nm VS. Molar Concentratio.

the nitrosophenyl compound (12,3). According to the Beer's law relationship, a plot of absorbance versus molar concentration should give a straight line that passes through the origin. The slope of this line corresponds to the molar absorptivity (13). Table 1 shows the molar absorptivity values for the two molecules (time zero = reduced form, E^R; time infinity = oxidized form, E^O).

The molar absorptivity is independent of path, concentration or light intensity, but is dependent on the solvent, wavele-

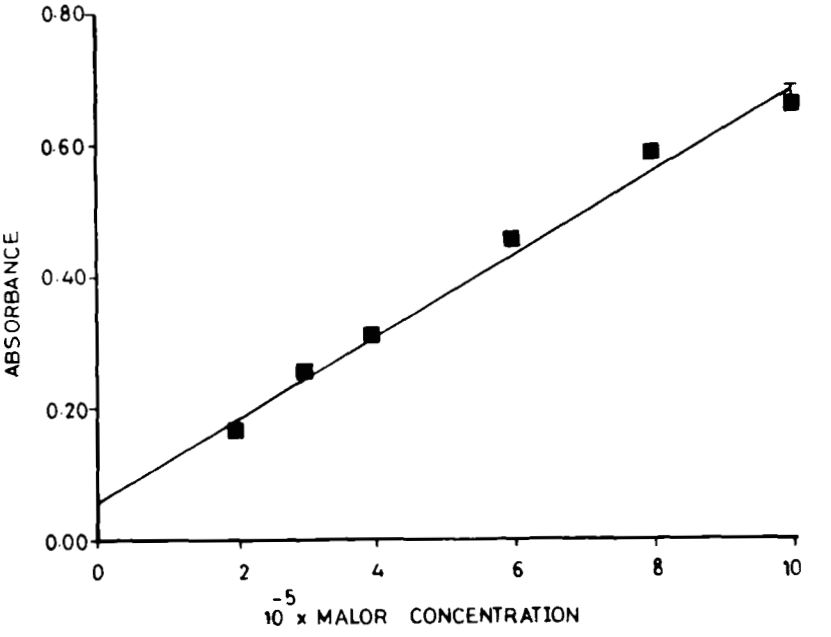

FIGURE 5

Plot of Absorbance of the Oxidized from of Nifedipine at 280 nm VS. Molar Concentration.

TABLE 1

Molar Absorptivity Values of Nifedipine (E^R) and its Oxidized Form (E^O) at Different Wavelengths.

E	E^R_{237}	E^R_{280}	E^R_{360}	E^O_{237}	E^O_{280}	E^O_{360}
Value X10^4	1.57	0.23	0.36	1.02	0.63	0.10

ngth, temperature and to a lesser extent other experimental variables.

Each data point in the graphs represents the mean of three determinations and show a very low standard error of the mean. The slope of each represents the line of best fit with a correlation coefficient greater than 0.991 and a standard error of estimate less than 0.05. These indicate that our molar absorptivity values are reproducible with a very low percent of error. The lines did not pass through the origin. This may be due to some impurity in the sample.

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