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Original Article

Effect of Microencapsulation on Photo-Stability of Nifedipine

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Abstract

Nifedipine (NIF), a 1,4-dihydropyridine calcium channel antagonist, undergoes photo-degradation to dehydro-nifedipine (DNIF) upon exposure to ultraviolet (UV) light and to the nitroso analogue of dehydro-nifedipine (NDNIF) when exposed to sunlight or some kinds of artificial lights. NIF photo-degradation products do not contribute to clinical activity, thus prevention of photo-degradation of NIF formulations is very important. Large differences in photo-stability between bioequivalent NIF products could potentially result in the therapeutic failure of unstable preparations. The aim of this study was to evaluate the effect of microencapsulation on nifedipine photo-stability. Four different microspheres of nifedipine were prepared using ethyl cellulose, ethyl cellulose plus titanium oxide, pectin and gelatin. Microspheres were exposed to fluorescent light and the content of NIF, DNIF and NDNIF for each product was measured using a specific and sensitive reversed phase high-pressure liquid chromatography (HPLC) method to determine the extent of photo-decomposition. In addition, photo-degradation of pure NIF powder was compared with acidic and buffer solution of NIF. Solution of NIF degraded in one day, while microencapsulation of NIF prevented the photo-degradation for up to six days against light exposure. Therefore, it may be concluded that present microencapsulation method without using other compounds such as opaque materials do not provide enough protection.

Keywords: Nifedipine; Photo-stability; HPLC; Microsphere; Ethyl cellulose; Pectin, Gelatin.

Introduction

Nifedipine, 1, 4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-3, 5-pyridine dicarboxylic acid dimethyl ester (Figure . 1A), is the prototype compound of the dihydropyridine class of calcium channel antagonists. NIF is a selective arterial dilator, and is frequently used for the treatment of hypertension, angina pectoris and other cardiovascular disorders (1). In human, NIF is rapidly metabolized by oxidative mechanisms to dehydro-nifidipine (DNIF), (Figure . 1). In case of exposure to light, it is further metabolized to more polar compounds (2-6).

NIF is highly sensitive to photo-oxidation, changing in color from yellow to brown upon exposure to light. NIF is degraded to DNIF and the nitroso-analogue of dehydro-nifedepine (NDNIF) (Figure . 1) (7). NIF photo-degradation products have little or no pharmacological activity (8-9). Therefore, quantitative analysis of NIF formulations requires the accurate detection and quantification of NIF and its major photo-decomposition product NDNIF. Few quantitative studies on the light transmissive properties of different light protective tablet film coatings or protective packaging have previously been

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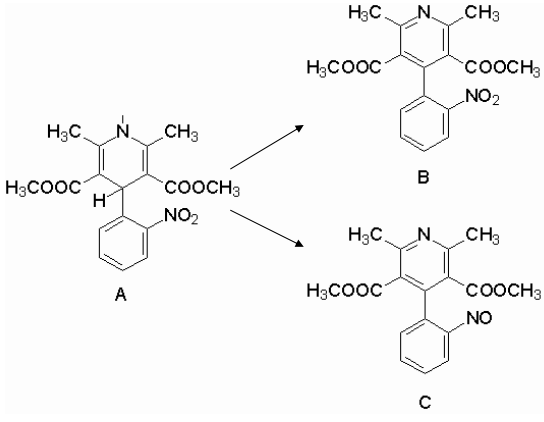

Figure 1. Chemical structures of nifedipine (A), nitro-analogue of dehydronifedipine (B) and nitroso-analogue of dehydronifedipine (C).

carried out (10). Thus, difference in degree of light protection may exist between different brands and /or formulation types of NIF products. Long term exposure, several weeks or longer, to direct sunlight may occur if NIF formulations are improperly stored by patients. Inappropriate storage conditions may potentially contribute to a decrease in clinical efficacy of NIF products.

The purpose of this study was to evaluate the effect of microencapsulation on photo-stability of NIF. Four different types of nifedipine microspheres were prepared and the quantity of NIF, DNIF and NDNIF were determined after light exposure at different time intervals. Also the degradation of NIF in solid and solution states were compared.

Experimental

Materials

NIF (USP), NDNIF (nitroso-phenyl-pyridine) and DNIF (nitro-phenyl-pyridine) from FIS (Italy) were kindly donated by Tolidaru, Iran. Oxazepam (USP), as the internal standard, was kindly provided by Sobhan Pharma. Co., Iran. Methanol, Poly vinyl alcohol (MW 72000), methylene chloride, gelatin, potassium dihydrogen phosph ate, titanium oxide, 2-propanol, glutaraldehyde 25%, toluene, HCl 37% were purchased from M erck (Germany). Pectin was purchased from CP Kelco (Denmark) and Sesame oil was obtained from Aseel (Emirate). Ethyl cellulose 40 cps (EC) from the Nippon Soda (Japan) was kindly donated by Sobhan Pharma Co, Iran.

Microspheres preparation

Three different polymers were used for the preparation of NIF microspheres:

1- Ethyl cellulose microspheres were prepared by solvent evaporation method (11). Nifedipine and ethyl cellulose with a total weight of 1000 mg were dissolved in 10 ml methylene chloride as the internal phase. Microspheres were prepared with three different drug to polymer ratios: 10%, 30% and 50%. The internal phase was then added drop-wise to a 0.5% w/v solution of poly (vinyl alcohol) (PVA) in water. The mixture was constantly stirred at 500 rpm using an overhead stirrer (Heidolph, Germany) up to 5 hours for complete evaporation of methylene chloride. Microspheres were then filtered and rinsed three times with distilled water and dried at room temperature.

2- Ethyl cellulose microspheres of NIF containing titanium oxide were also prepared in a similar way. 1, 5 or 10% w/w titanium oxide (in respect to EC) was added to the internal phase.

3- Gelatin microspheres of NIF were prepared by a suspension polymerization technique using glutaraldehyde as the cross-linking agent. 100mg nifedipine and 900 mg gelatin were dissolved in 10 ml water as the internal phase. The solution was added drop-wise to 50 ml sesame oil as the external phase while being stirred using an overhead stirrer at 300 rpm. 20 ml glutaraldehyde saturated toluene was then added to harden the microspheres. The cross-linked and hardened microspheres were washed with acetone, separated by filtration and dried overnight.

4- To prepare pectin microspheres, 100 mg NIF and 900 mg pectin were dissolved in 10 ml distilled water. This mixture was then completely degassed under vacuum (Fast VacTM, J/B Industries, USA). An electrostatic bead generator (Nisco encapsulator VAR V-1, Switzerland) equipped with a syringe pump (KD Scientific, USA), was employed to prepare the beads by ionotropic gelation. The mixture was dropped into the cross-linking solution of zinc acetate, at the rate of 10 ml/h. The cross-linked pectin beads were washed twice with distilled water and dried overnight at room temperature.

All microsphere preparation procedures were conducted at room temperature under sodium

lamp to protect NIF from photo-degradation.

Irradiation test

The irradiation test was employed utilizing a 26 Watt ballast lamp placed 20 cm above the samples. Nifedipine microspheres were placed on an aluminum foil to allow uniform irradiation. Irradiation was conducted inside a dark room with controlled temperature to protect samples from extraneous light. Aliquots for analysis were taken at days 0, 1, 2, 3 and 6.

All experiments were performed in triplicate and the mean ± SD were reported.

Amount of NIF, DNIF and NDINF in the microspheres before and after light exposure were measured using a reversed phase HPLC method (12). Briefly, a C8 column (Nova-Pack Waters, Milford, MA, 8×100 mm, 4µm) and a tertiary mixture of acetonitril:methanol: water (25:50:25) were used as stationary and mobile chromatographic phases, respectively. A pump-controller unit (Knauer, K-1001, Berlin, Germany) and a rheodyne injection device (Knauer, D-14163, Berlin, Germany) equipped by a 20µl loop were used for solvent delivery (FR: 1 ml/min) and sample injection respectively. Appropriate amounts of nifedipine microspheres were used for NIF extraction. Methanolic solutions of NIF containing known amount of oxazepam as internal standard were used for NIF and DNIF determination. Sample spectra were recorded using Eurochrom HPLC software version 2.05 on a NEC computer. The UV/VIS absorbance detector was set at 235 nm.

Calibration curves were constructed by plotting the peak areas versus their corresponding added concentrations. Concentrations were in the range of 10 to 200 µg/ml for NIF and 1 to 100 µg/ml for DINF and NDINF. An un-weighed least squares linear regression analysis was performed to generate a best-fit regression line for each compound.

Results and Discussion

Figure 2 shows the SEM photographs of different microspheres prepared in this study. As can be seen, ethyl cellulose microspheres had smooth surface and spherical shape. However, both gelatin and pectin microspheres were non-spherical with rough surface which could be attributed to the changes made by drying. Because of the hydrophilic nature of these two polymers, they absorb water and swell during the microsphere preparation procedure. These swelled microspheres lose their water content during drying time and the collapsed microspheres are formed.

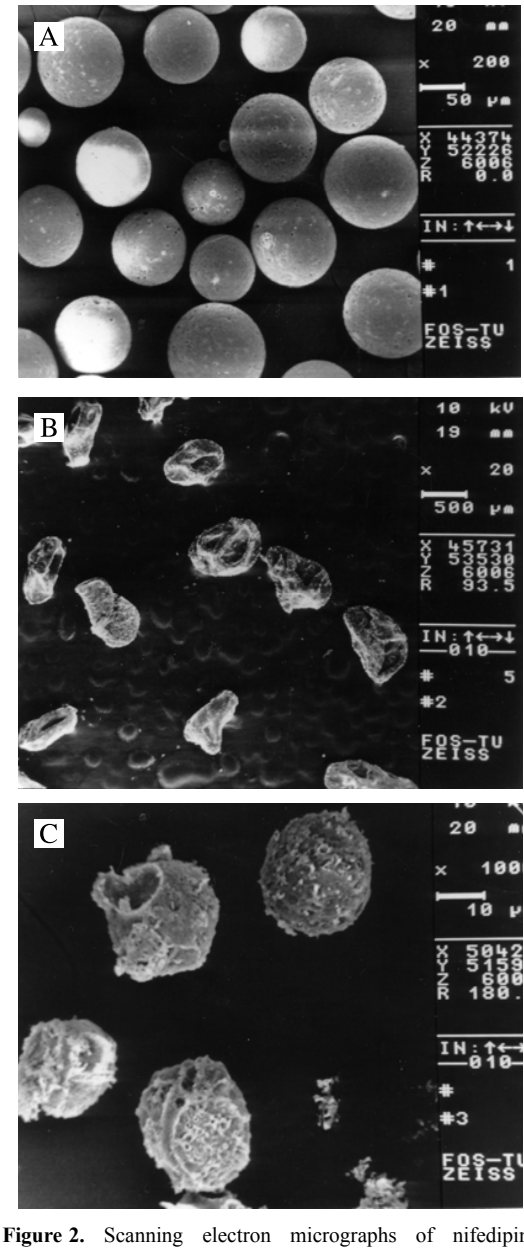

Figure 2. Scanning electron micrographs of nifedipine microspheres prepared with different polymers: A) Ethyl cellulose, B) Pectin and C) Gelatin.

Table 1 shows the microsphere preparation yield and the drug loading efficiency. Ethyl cellulose and gelatin microspheres showed the microencapsulation yield higher than

Table 1. Microencapsulation yield and drug loading efficiency of different microsphere formulations.

formulation	Microencapsulation yield (%)	Drug loading efficiency (%)
Ethyl cellulose (10% drug)	83	72
Ethyl cellulose (30% drug)	85	95
Ethyl cellulose (50% drug)	82	99
Gelatin (10% drug)	88	42
Pectin (10% drug)	60	44
Ethyl cellulose-Titanium oxide (99:1), (10% drug)	56	82
Ethyl cellulose-Titanium oxide (95:5) (10% drug)	55	75
Ethyl cellulose-Titanium oxide (90:10) (10% drug)	56	78

80%. Combination of titanium oxide with EC, decreased the microencapsulation yield. This value was around 60% for pectin microspheres. Drug loading efficiency was more than 70% for all the formulations prepared using EC.

A typical chromatograms of NIF and its photo-degradation products is shown in Figure 3. As can be seen, the peaks of NIF, nitro and nitroso-analogue were completely separate and no interference was observed between any of the compounds tested.

Table 2 compares the ratio of nitroso analogue to initial NIF content on days 1 and 3 in different microsphere formulations. The lowest ratio was obtained for pectin and ethyl cellulose with titanium oxide that means the higher photo-protection ability of these polymers.

Figure 4 shows the photo-degradation of NIF in the form of powder, buffer or acidic solution as well as microsphere formulation. The highest photo-degradation rate was observed in soluble form. There was no significant difference between buffer or acid solution. While both acidic and buffer solutions of nifedipine degraded within one day of light irradiation, 80% of nifedipine content of ethyl cellulose and titanium oxide microspheres remained intact. Nifedipine molecules in soluble form are more exposed to light and degrade faster.

In the other part of present study, microspheres were prepared using different ratios of ethyl cellulose to NIF. Figure 5 shows that the ratio of ethyl cellulose in microspheres had no significant effect on photo-stability of drug. All formulations prepared with EC:NIF ratios of 90:10, 70:30 and 50:50 lost all their nifedipine content after one day light exposure. It is evident that ethyl cellulose could not provide enough protection for nifedipine against light

Table 2. Ratio (%) of nitroso analogue to initial NIF content on days 1 and 3 in different microsphere formulations after light exposure.

formulation	NDNIF/initial NIF ratio (%)	
	Day 1	Day 3
Ethyl cellulose (10% drug)	98	100
Ethyl cellulose (30% drug)	91	100
Ethyl cellulose (50% drug)	85	99
Gelatin (10% drug)	69	75
Pectin (10% drug)	12	49
Ethyl cellulose-Titanium oxide (99:1), (10% drug)	23	77
Ethyl cellulose-Titanium oxide (95:5) (10% drug)	23	81
Ethyl cellulose-Titanium oxide (90:10) (10% drug)	19	83

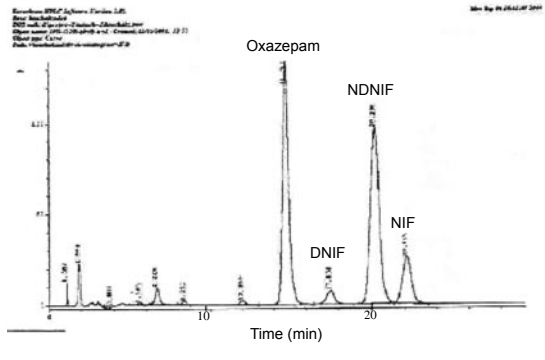

Figure 3. Chromatogram of nifedipine and its photo-degradation products (DNIF and NDNIF) days 1 and 3 in different microsphere formulations after light exposure.

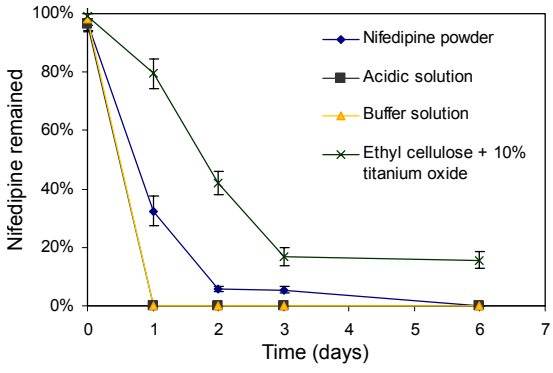

Figure 4. comparison of the photo-stability of nifedipine in the form of powder, acidic or buffer solution and microsphere.

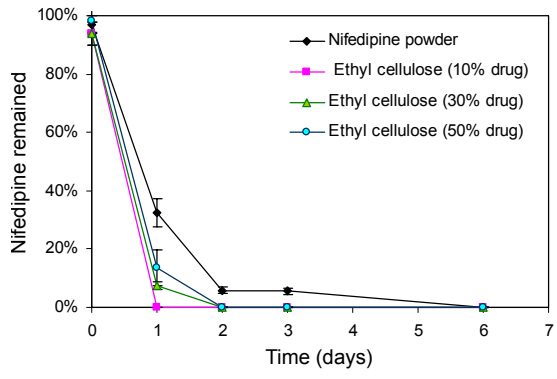

Figure 5. Effect of different amounts of ethyl cellulose on nifedipine degradation.

degradation. Unexpectedly, microencapsulation of NIF using EC decreased the photo-stability in compare with NIF powder. This effect could be explained by physical characteristic of powder and EC microspheres. Microencapsulation of adhesive NIF powder changed it to a free flow product, which provided a better light exposure for NIF molecules. But in the case of row powder, because of the adherence of drug particles together, light could penetrate into the NIF aggregates in less extents and some parts of drug particles remained intact.

However combination of EC with an opaque material like titanium oxide improved the photo-stability of NIF and about 20% of NIF remained intact inside the microspheres after 6 days of light irradiation (Figure 6). As it is shown in Figure 7, This protective effect was the same for different ratios of EC : titanium oxide (99:1, 95:5 and 90:10). Aman and Thoma studied the effect of titanium oxide for photo-stabilizing of molsidomine tablets. They reported that photo-stabilization by adding 0.5% titanium oxide was noticeable but further increase of the pigment content had no more effect (13).

Figure 8 shows the effect of different polymers used for microencapsulation on nifedipine photo-stability. Among four formulations, microspheres prepared with pectin provided the highest photo-protection for nifedipine. The photo-protection ability of polymers was in the following order:

Pectin > ethyl cellulose + titanium oxide > gelatin > NIF powder > ethyl cellulose

It can be said that polymeric films can only provide for the protection of photo sensitive substances when the polymer prevents the light penetration. This may be the reason that titanium oxide prolongs the photo-stability of nifedipine.

Conclusion

According to results obtained in this study, neither of microspheres provided enough protection of nifedipine from photo-degradation.

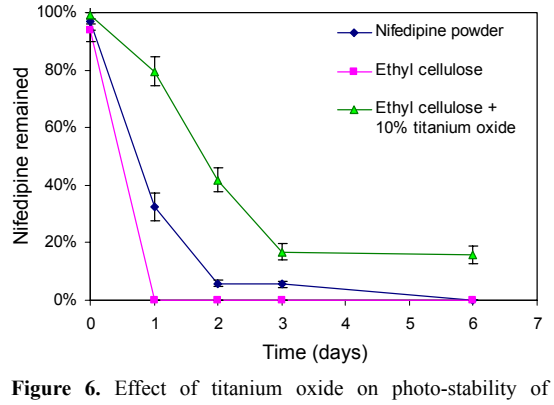

Figure 6. Effect of titanium oxide on photo-stability of nifedipine in ethyl cellulose microspheres.

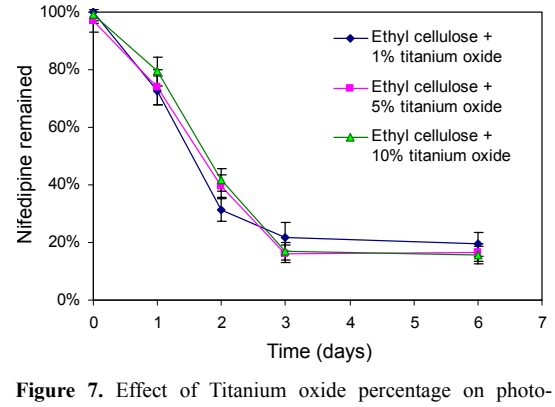

Figure 7. Effect of Titanium oxide percentage on photo-stability of nifedipine in ethyl cellulose microspheres.

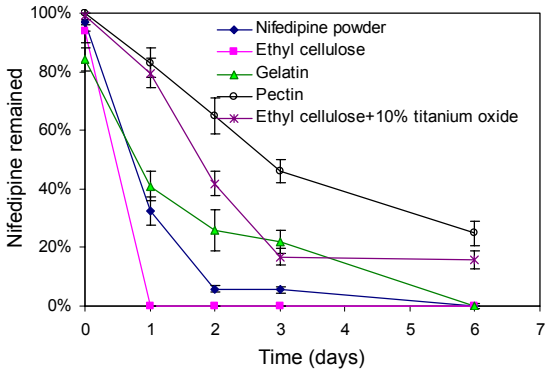

Figure 8. Effect of different polymers used for microencapsulation on nifedipine photo-stability

However microspheres prepared with pectin and ethyl cellulose containing titanium oxide protected nifedipine from photo-degradation for up to 6 days of light exposure.

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