

Research Paper

FTIR SPECTROSCOPIC INVITRO DRUG INTERACTION
STUDY OF NIFEDIPINE MICROSPHEREPATITAPABANA PARIDA¹, SUBASH CHANDRA MISHRA²,
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Nifedipine acts as a calcium channel blocker which shows therapeutic values against Hypertension, Angina, and other Cardiac diseases. The present study is to investigate the possibility of developing fingerprinting characteristic of Nifedipine Microsphere by using combined polymer (Ethylcellulose, Polyvinylalcohol) in different proportions required in the formulation of Microsphere for Sustained Release Medication. Solvent evaporation method was used for preparation of Microsphere containing Nifedipine, taking Ethyl cellulose as polymer. Identification of functional group frequencies with the help of Fourier Transform Infrared Spectroscopy has been made. Microspheres are prepared by taking drug to polymer weight ratio of 10:90. From FTIR (400 cm⁻¹ to 4000 cm⁻¹ region) spectroscopic studies were carried out and spectra were used for identification for in-vitro interaction study. It has been found that in Nifedipine Microsphere containing the Dihydropyridine nucleus of the Drug remains unchanged after the formulation which was improved the basic therapeutic activity with sustained release preparation.

KEYWORDS: Hypertension, Nifedipine, Ethylcellulose, Microsphere, FTIR, Interaction study**INTRODUCTION**

Nifedipine is a prototype Dihydropyridine calcium channel blocker with a rapid onset and short duration of action, IUPAC name being [Dimethyl-1, 4-dihydro-2, 6-dimethyl-4(2-nitrophenyl)-3, 5-pyridine carboxylate]¹. Inhibiting passage of calcium through the voltage gated L-type (for Large/Long-lasting current) calcium channel on vascular smooth muscle cells and cardiac myocytes, reducing calcium availability for muscle contraction. Depolarization of the tubule membrane activates the longitudinal sarcoplasmic reticulum via dihydropyridine receptors. These receptors are voltage gated Ca²⁺ channels in the t-tubule membrane and are so called because they get blocked by the drug dihydropyridine^{2,3}. Dihydropyridine an inhibitor of the T-type (Transient current) cardiac calcium channel^{1,4}. The overriding action of Nifedipine is arterial dilation i.e. total peripheral resistance decrease, BP falls the direct depressant action on heart require much higher dose, but a weak negative inotropic action can be unmasked after beta blockage. Nifedipine doesn't depress SA or AV node^{5,6}. Nifedipine Has mild natriuretic action but significant diuresis does not occur⁷. The dihydropyridines have much less effect on the cardiac tissues and higher specificity for the arteriolar vascular bed. Dihydropyridines are used more frequently as anti-anginal and anti-hypertensive agents. It may be preferred over Nifedipine for patients with angina pectoris or hyper tension who also have CHF dysfunction. It was indicated that Low Solubility and High Permeability of Drug in-vivo⁷. There have been multiple reports in the medical literature of serious adverse effects with sublingual Nifedipine, including cerebral ischemia/infarction, myocardial infarction, complete heart block, and death². Many medications such as peptide and protein, antibody, vaccine and gene based drugs, in general may not be delivered using these routes because they might be susceptible to enzymatic degradation or can not be absorbed into the systemic circulation efficiently due to molecular size and charge issues to

be therapeutically effective. For this reason many protein and peptide drugs also have to be delivered by injection or a nano/micro-needle array. For example, many immunizations are based on the delivery of protein drugs and are often done by injection.⁸

Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues) and sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation. Types of sustained release formulations include microsphere, drug loaded biodegradable microsphere and drug polymer conjugates. The goal of this piece of research work is to calibrate to identification of functional groups and nucleus of the core drug which was unchanged after the formulation and to detect the compatibility of the drug which shows in-vitro drug interaction.

MATERIALS AND METHODS**Chemicals Used**

Nifedipine (Gift from J.B Pharma), Ethyl Cellulose (Merck), Polyvinylalcohol (S.D. fine), Dichloromethane, KBR (Finar), Distilled water from Shneltsu Distillation Instrument.

Methods:

Ethyl cellulose Microspheres were prepared by solvent evaporation method. Nifedipine and ethyl cellulose with a total weight of 1000 mg were dissolved in 10 ml Ethylene chloride (dichloromethane) as the internal phase. Microspheres were prepared with three different drugs to polymer ratios: 10%, 20% and 30%. 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 (Remi) 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.^{08 to 14}

Evaluation of Microsphere

Instrumentation

Perkin Elmer Spectrum RX (1)

The FTIR imaging in the present investigation was carried out using an interfaced with infrared (IR) microscope operated in reflectance mode. The microscope is equipped with a video camera, a liquid Nitrogen-cooled Mercury Cadmium Telluride (MCT) detector and a computer controlled translation stage, programmable in the x and y directions. Here KBr pellet method was used for sample preparation for FTIR study. The spectra were collected in the 400 cm⁻¹ to 4000 cm⁻¹ region with 8 cm⁻¹ resolution, 60 scans and beam spot size of 10 μm-100 μm. The results are analyzed below and compared.

Procedure

FTIR Spectroscopic Analysis

A KBr pellet was prepared by grinding the solid sample with solid potassium bromide (KBr) and applying great pressure to the dry mixture. KBr is chosen because it is transparent to infrared Radiation. If the pellet is prepared properly, one can actually see through it, as through a pane of glass. 2 mg of each drug sample were taken with dry IR-grade KBr at about 2% sample to KBr ratio in an agate mortar. The grinding was carried out until it was uniformly distributed throughout the KBr. Some amount of the mixture was transferred to the pellet making die and by applying as small pressure to the die before pulling the vacuum. Then full pressure of 10,000 pounds to 16,000 pounds was applied to the die for 2 min. First vacuum was released then pressure. KBr pellet of the drug sample was prepared. Then a vacuum was pulled for 1 to 2 min.

The die set was disassembled by removing the base by twisting it off and releasing the 'O' ring seal. The pellet was discharged with the clear cylindrical pellet extractor located above the end of the bore and the plunger located beneath the assembly. Normally background was first scanned by using blank potassium bromide pellet. Then the sample was scanned. The spectra were collected in the 400 cm⁻¹ to 4000 cm⁻¹ region with 8 cm⁻¹ resolution, 60 scans and beam spot size of 10-100 μm^{15, 16}.

RESULTS AND DISCUSSION

The infrared spectra are recorded on Fourier Transform Spectrometer in the mid-infrared region (MIR) within the range (400-4500 cm⁻¹). Due to the complex interaction of atoms within the molecule, IR absorption of the functional groups may vary over a wide range. However, it has been found that many functional groups give characteristic IR absorption at specific narrow frequency range. Multiple functional groups may absorb at one particular frequency range but a functional group often gives rise to several characteristic absorptions. Stretching (ν) & bending vibrations are varied after formulation can be observed. Thus, the spectral interpretations should not be confined to one or two bands only actually the whole spectrum should be examined^{16 to 22}.

1. RESULTS OF FTIR STUDY: NIFEDIPINE (THE PURE DRUG)

While the FTIR band at 4000-1300 cm⁻¹ represent the functional group region, the appearance of strong

absorption bands in the region of 3400 cm⁻¹ is due to stretching vibrations of N-H free, stretching of Ar-H, (-CH) several band in the region of 3096 cm⁻¹. 2872 cm⁻¹ shows methyl group where C-C symmetric, in the region of 1680 cm⁻¹, is due to C=O stretching vibration. Pyridine nucleus ring breathing in the region of 1622 cm⁻¹. Aryl nitro compound are found in the region of 828 cm⁻¹ shows C-N stretching vibration. (fig.1)

2. RESULTS OF FTIR STUDY: ETHYLCELLULOSE

While the FTIR band at 1376 cm⁻¹ represented O-H group region, the appearance of strong absorption bands. In the region of 1114 cm⁻¹ is due to stretching vibrations of C-O-C very strong stretching, bending of (-CH) shows presence of Etherial group. (fig.2)

3. RESULTS OF FTIR STUDY: POLYVINYL ALCOHOL

While the FTIR band at 3562 cm⁻¹ represented O-H group region, the appearance of strong absorption bands and stretching of O-H, H-bonded single bridge. In the region of 2970 cm⁻¹ due to stretching vibrations of C-H, 2 or 3 band represents methyl group. (fig.3)

4. RESULTS OF FTIR STUDY: NIFEDIPINE + ETHYLELLULOSE

The FTIR band at 3450 cm⁻¹ represented O-H group stretching of O-H, H-bonded single bridge. In the region of 3086 cm⁻¹ is due to stretching vibrations of Ar-H, (-CH) several band at 2972 cm⁻¹ (C-H), 2 or 3 band of methyl group. The presence of aryl carboxylic group in the region 1678 cm⁻¹ represents C=O stretching vibration. 1624 cm⁻¹ represents presence of pyridine nucleus ring breathing. Presence of aryl nitro group in the region of 1555-1487 cm⁻¹ is also observed. Etherial group is found at 1152 cm⁻¹, where C-O-C shows very strong stretching. (fig.4)

1. RESULTS OF FTIR STUDY: MICROSPHERE WITHOUT DRUG

The FTIR band at 2974 cm⁻¹ represents C-H stretching; 2 to 3 band is for presence of methyl group. In the region of 1378 cm⁻¹ were due to bending vibrations of δ O-H and stretching vibration of C-O and C-O-C very strong stretching of (-CH₂-O-CH₂-) at 1120 cm⁻¹ shows presence of etherial group. (fig.5)

6. RESULTS OF FTIR STUDY: NIFEDIPINE MICROSPHERE

While the FTIR band at 2876 cm⁻¹ represented methyl group stretching of CH₃ more than one band, C-C symmetric. In the region of 1990 cm⁻¹ were due to bending vibrations of Ar-H, out of plane substitution, C=O strong stretching at 1696 cm⁻¹ shows presence of aryl carboxylic group. Pyridine nucleus is found in the region of 1622 cm⁻¹ Ring vibration (Ring breathing) band. 1470 cm⁻¹ represents presence of hydroxyl group bending of O-H and stretching of C-O 1555-1487 cm⁻¹. Ester group is found at 1242 cm⁻¹ where C-O-C stretching and finally presence of aryl nitro group in the region of 844 cm⁻¹ where medium to strong stretching of -N=O. (fig.6)

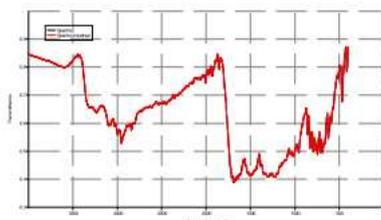


Fig-1: FTIR Spectra of Nifedipine

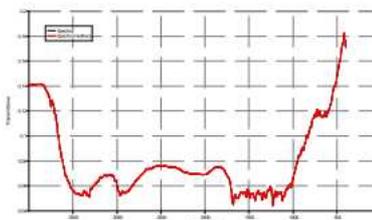


Fig-4: FTIR Spectra of Nifedipine + Ethylcellulose

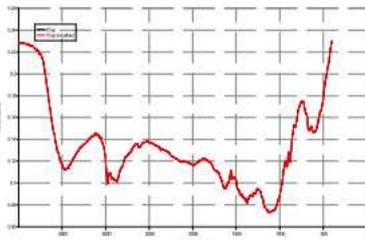


Fig-2: FTIR Spectra of Ethylcellulose

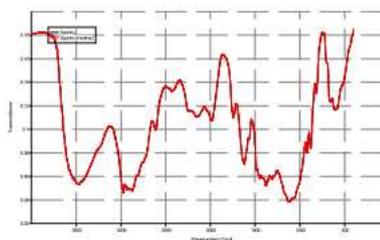


Fig-5: FTIR Spectra of Microsphere without Drug

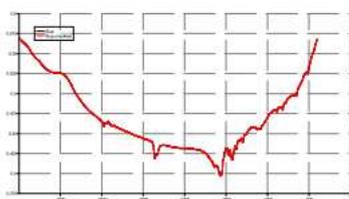


Fig-3: FTIR Spectra of Polyvinyl alcohol

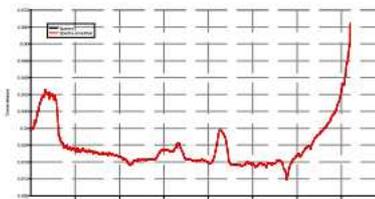


Fig-6: FTIR Spectra of Nifedipine Microspheres

2.3. COMPARATIVE STUDY OF FTIR PEAKS

2.3.1. Prominent FTIR Peaks of Nifedipine

PEAKS (cm ⁻¹)	GROUPS (gr.)	PEAK ASSIGNMENTS
3400	Amine gr.	ν (N-H) free, trans-isomer
3096	Aromatic gr.	ν Ar-H (-CH) several band
2872	Methyl gr.	ν (CH ₃) C-C symmetric, more than one band
1680	Aryl Carboxylic gr.	ν C=O Stretching Vibration
1622	Pyridine gr.	Ring vibration (Ring Breathing) band
1226	Carbonyl gr.	ν C-O / δ O-H
828	Aryl Nitro gr.	ν C-N stretching vibration

2.3.2 Prominent FTIR Peaks of Ethylcellulose

PEAKS (cm ⁻¹)	GROUPS (gr.)	PEAK ASSIGNMENTS
1376	Hydroxyl gr.	δ O-H, ν C-O
1114	Etherial gr.	ν C-O-C very strong stretching
830	Etherial gr.	δ C-H cis-epoxide

2.3.2 Prominent FTIR Peaks of Polyvinyl alcohol

PEAKS (cm ⁻¹)	GROUPS (gr.)	PEAK ASSIGNMENTS
1376	Hydroxyl gr.	δ O-H, ν C-O
1114	Methyl gr.	ν C-H, 2 or 3 band

2.3.2 Prominent FTIR Peaks of Nifedipine + Ethylcellulose

PEAKS (cm ⁻¹)	GROUPS (gr.)	PEAK ASSIGNMENTS
3450	Hydroxyl gr.	ν O-H, H-Bonded, single bridge
3086	Aromatic gr.	ν Ar-H, (-CH) several band
2972	Methyl gr.	C-H, 2 or 3 band
1678	Aryl Carboxylic gr.	ν C=O stretching Vibration
1624	Pyridine gr.	Ring vibration (Ring Breathing) band
1555-1487	Aryl Nitro gr.	ν C-N stretching vibration
1152	Etherial gr.	ν C-O-C very strong stretching

2.3.2 Prominent FTIR Peaks of Microsphere without Drug

PEAKS (cm ⁻¹)	GROUPS (gr.)	PEAK ASSIGNMENTS
2974	Methyl gr.	ν C-H, 2 to 3 band
1378	Hydroxyl gr.	δ O-H/ ν C-O
1120	Ethereal gr.	ν C-O-C, VERY STRONG stretching

2.3.6. Prominent FTIR Peaks of Nifedipine MICROSPHERE

PEAKS (cm ⁻¹)	GROUPS (gr.)	PEAK ASSIGNMENTS
2876	Methyl gr.	ν CH ₃ more than one band, c-c symmetric
1990	Aryl gr.	δ Ar-H, out of plane substitution
1696	Aryl Carboxylic gr.	ν C=O strong stretching
1622	Pyridine nucleus	Ring vibration (Ring breathing) band
1470	Hydroxyl gr.	δ O-H/ ν C-O
1242	Ester gr.	ν C-O-C stretching
844	Aryl gr.	ν -NO medium to strong stretching

CONCLUSIONS:

The study based on analytical characterization of Microsphere containing Nifedipine the Cardiac Drug. The Infrared Spectroscopic data shows that there is no more Drug interaction takes place in between Dihydropyridine nucleus of the Drug & Ingredients/polymers used for Sustained release formulation which is identified by Infrared Spectroscopic Analysis. The Dihydropyridine ring and other functional groups are unaffected after the formulation which shows the therapeutic activity as referred to the qualitative structural activity taken to consideration. And in-vitro stability is not hampered after formulation.

NOMANCLATURE

Stretching Vibration	ν
Bending Vibration	δ
Strong Intensity	str
Weak Intensity	w
Strong to weak Intensity	s-w
Medium Intensity	med
Strong-Medium Intensity	s-m
Variation Intensity	var

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