ATR-FTIR Based Pre and Post Formulation Compatibility Studies for the Design of Niosomal Drug Delivery System Containing Nonionic Amphiphiles and Chondroprotective Drug

[1]Muhammad Imran Khan, [1]Asadullah Madni*, [1]Saeed Ahmad, [2]Ahmad Khan
[1]Mubashar Rehmanand [1]Muhammad Ahmad Mahmood
[1]*Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan.*
[2]*Department of Pharmacy, Quaid-e-Azam University, Islamabad 45320, Pakistan.*
asadpharmacist@hotmail.com

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Summary: Pharmaceutical compatibility studies are considered as the most important and first screening stage during development of pharmaceutical drug product. Attenuated total reflectance/fourier transform infrared (ATR-FTIR) is one of the techniques currently available to pharmaceutical scientists for investigating the compatibilities between active drug and inactive pharmaceutical ingredients. The present study was designed to assess the interaction among different niosomes forming components i.e nonionic amphiphiles and chondroprotective/antiinflamatory drug Diacerein by ATR-FTIR method. Physical mixtures and niosomes were prepared by physical mixing and thin film hydration method, respectively. The individual niosomal components, physical mixtures as well as niosomal formulations were analyzed. The spectra of Diacerein showed characteristic peaks at 3300 cm^{-1} (–COOH) and 760 cm^{-1} (m-substituted benzene), Span 60 at 2916 cm^{-1} (–OH), Span 80 at 1740 cm^{-1} (5- membered ring), Span 85 at 1643 cm^{-1} (ketone with 5-membered ring), Tween 20 at 1734 cm^{-1} (5-membered ring) and Tween 80 at 3488 cm^{-1} (–OH). The characteristic peaks of Diacerein were present in niosomal formulations with slight shift at 3355-3379 cm^{-1} (–COOH) and 760-770 cm^{-1} (m-substituted benzene). This work suggested no significant interaction in characteristic peaks of Diacerein after combining with nonionic surfactants as physical mixtures and niosomal formulations which proposed potential for niosomes to encapsulate diacerein in their micro vicinity.

Keywords: Compatibility, ATR-FTIR, Diacerein, Physical Mixtures, Niosomes, Nonionic surfactants.

Introduction

The interaction studies of active and inactive pharmaceutical ingredients are important step in preformulation phase of product development. The physical and chemical incompatibilities between drugs and excipients can alter the chemical nature, bioavailability and stability profile of drugs and ultimately their therapeutic effectiveness and safety [1]. A formulation is regarded as acceptable when no drug-excipient or excipient-excipient incompatibility occurs. Keeping in view such prerequisites, designing a quick and accurate method to test and select the best excipients for stable dosage form becomes a goal in preformulation studies [2].

ATR-FTIR is a very useful tool in fingerprint identification of active moiety and excipient molecules. Distinct characteristics IR bands of different molecules provide the ability to identify specific functional groups of the structural components constituting different pharmaceutical dosage forms. In addition, the possible relationship between IR absorbance and the concentration facilitates the quantitative estimation of individual components of the formulation [3]. This technique is able to probe the *in situ* single or multiple layers of deposited/adsorbed species at solid/liquid interface. Thus, ATR-FTIR has been applied extensively in different biological studies with an aim to investigate possible chemical interactions at the solid/liquid interface [4].

Diacerein is a novel NSAID with several pharmacological actions such as analgesic, anti-inflammatory and antipyretic activities and gradually improves disease conditions owing to its IL-1β inhibitory action. Diacerein (9,10-Dihydro-4,5-dihydroxy-9,10-dioxo-2-anthroic acid diacetate) is purified anthraquinone derivative as shown in Fig. 1 which is converted to active metabolite rhein (1,8-dihydroxy-3-carboxyanthraquinone) [5, 6]. Its mechanism of action is different from other NSAIDs as it inhibits intracellular pathways and cartilage degradation in osteoarthritis (OA) and donot interefer with arachidonic acid. Therefore, diacerein is better tolerated for gastric and renal toxicity [7, 8]. Recently, different research groups reported clinically useful work on diacerein for the treatment of OA. Considering the damaging role of oxidative stress in OA, an anti-oxidant thymol was added as co-drug with diacerein and it offered promising

*To whom all correspondence should be addressed.

results [5]. Diacerein was also targeted to arthritic tissues with the help of chondroitin sulfate. Application of chondroitin sulfate, a soluble polysaccharide, showed better effects for the targeting of diacerein [9].

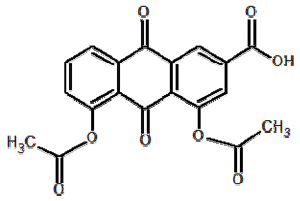

Fig. 1: Structural formula of Diacerein (9,10-Dihydro-4,5-dihydroxy-9,10-dioxo-2-anthroic acid diacetate)

Diacerein has low aqueous solubility and bioavailability in systemic circulation [10]. As niosomes are known to increase solubility and bioavailability of poorly soluble active moieties [11-13], hence for enhancing solubility profile of diacerein and its availability at site of absorption, various niosomal formulations have been prepared. The interaction studies provided a mean for evaluating the compatibility of the niosomal components with drug. Diacerein, physical mixtures of Diacerein with various non-ionic amphiphiles and developed niosomal formulations were analyzed by ATR-FTIR spectroscopic technique to investigate possible interactions.

Experimental

Materials

Diacerein was received as generous gift sample from Consolidated Chemical Laboratories (Pvt.) Ltd., Lahore, Pakistan. Sorbitan monostearate (Span 60), sorbitan monooleate (Span 80), sorbitan trioleate(span 85), polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene Sorbitan monooleate(Tween 80) were purchased from Merck Chemicals, Germany. Cholesterol was obtained from Sigma Aldrich. All the chemicals were of analytical grade.

Preparation of Physical Mixtures

Physical mixtures of drug and selected nonionic surfactants were prepared by mixing equimolar amounts of drug, cholesterol and nonionic surfactants in a glass mortar. Mixing was continued using pestle for about 10 minutes until homogeneity was achieved. The resultant PMs were designated as PM1, PM2, PM3, PM4, and PM5 [14].

Preparation of Niosomal Formulations

Representative niosomal formulations of Diacerein with various nonionic amphiphiles (Span 60, Span 80, Span 85, Tween 20, Tween 80) were prepared using thin film hydration method. It involves the mixing of drug, nonionic surfactant and cholesterol in chloroform/methanol (2:1). The solution was rotary evaporated at 60°C untill thin film was obtained on the wall of flask. Then this film was hydrated with phosphate buffer saline (pH 7.4) for 1 hr. Niosomal formulations were stored at 4°C to get mature. In order to maintain sterile conditions, all steps of preparation were carried out in aseptic conditions [15].

FTIR Spectroscopic Study by ATR Method

The spectra of Diacerein, cholesterol, non-ionic surfactants, physical mixtures and niosomal formulations were taken by scanning in the wave number range of 4000-400 cm^{-1} using FTIR spectrophotometer (Bruker Tensor 27 series, Germany). Pike single bounce attenuated total reflectance (ATR) method was adopted for scanning individual components, physical mixtures and niosomal formulations.

Results and Discussion

Fourier transform infrared spectroscopy (FTIR) is the most applied technique in the chemical and pharmaceutical sciences. Its scope includes many applications especially to find out the compatibility between active and inactive pharmaceutical ingredients, which is prerequisite for the designing of drug formulation. The FTIR spectra of individual components, physical mixtures and niosomal formulations were recorded and interpreted to find out any interaction. This work was aimed to point out presence or absence of any possible incompatibility of selected nonionic surfactants with diacerein.

FTIR Spectrum of Active Ingredient

The FTIR spectrum of Diacerein (Fig. 2) showed characteristic bands at 3300 cm^{-1} (–OH stretch broad, –COOH), 3069.20 cm^{-1} (C–H, stretch, aromatic), 2935 cm^{-1} (C–H, stretch, aliphatic, symetric), 1764.56 cm^{-1} (C=O, ester), 1677 cm^{-1} (C=O stretch COOH), 1593.20 cm^{-1} (C=C stretch aromatic), 1450.47 cm^{-1} (C–O stretch, COOH), 1024.21 cm^{-1} (C–O stretch, ester), 760.43 cm^{-1} (m-substituted benzene) and 703.02 cm^{-1} (benzene). These characteristic peaks are also observed

elsewhere in literature which indicates the purity of Diacerein and absence of any kind of impurity [16].

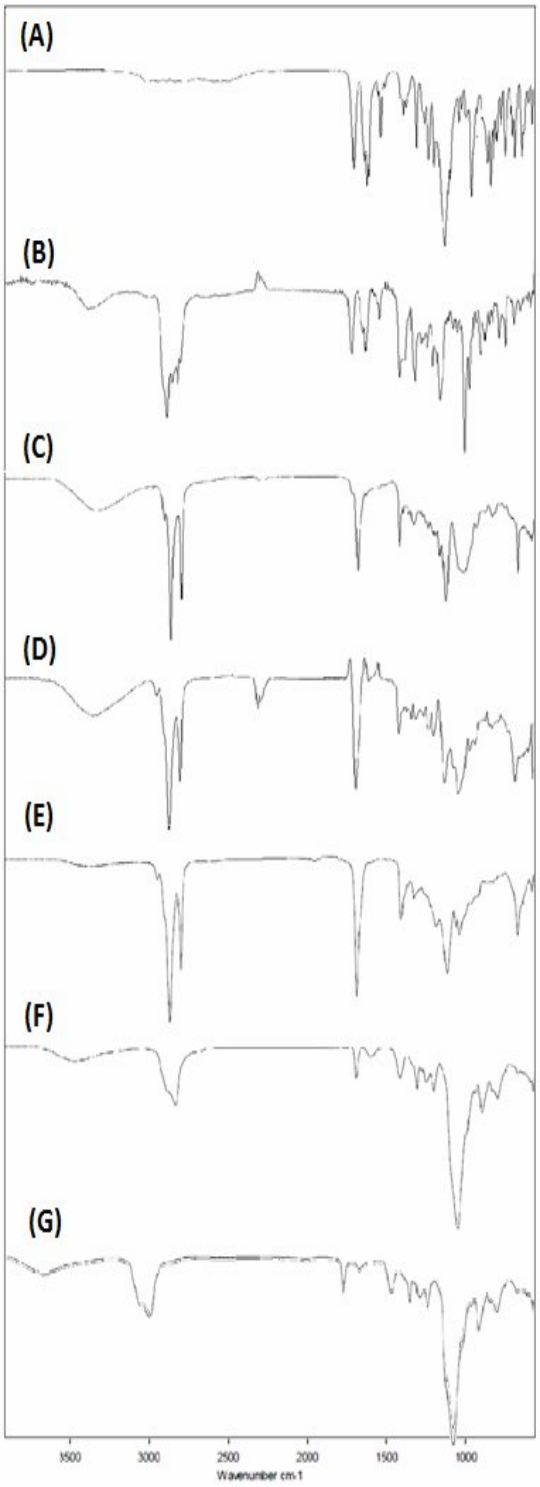

Fig. 2: FTIR Spectra of Individual components : (A) Diacerein, (B) Cholesterol (C) Span 60 (D) Span 80 (E) Span 85 (F) Tween 20 (G) Tween 80

FTIR Spectra of Excipients

Span 60 showed characteristic peaks at 2916.75 cm^{-1} (–OH str, broad), 2849.58 cm^{-1} (–OH str, broad), 1734.65 cm^{-1} (cyclic 5-membred ring), 1467.56 cm^{-1} ($–CH_3$), and small peaks 1000 cm^{-1} to 1200 cm^{-1} (aliphatic) [17]. Span 80 has shown characteristic peaks at 2412.63 cm^{-1} (aromatic ring), 2923 cm^{-1} (–OH group), 1740.15 cm^{-1}(presence of 5 membered ring), 1463.49 cm^{-1} ($–CH_3$), 1378.58 cm^{-1} ($–CH_3$ symmetrical deformable) and small peaks in the range of 1000 cm^{-1} (aliphatic) [18]. Span 85 has shown characteristic peaks at 2923.18 cm^{-1}, 2853.50 cm^{-1} both peaks represent (double bond $–CH_2$), 1743.05 cm^{-1} (ketone with 5 membered cyclic ring), 1463.44 cm^{-1} (scissoring double bond $–CH_2$), 1377.42 cm^{-1} ($–CH_3$), 1167.75 cm^{-1} (C–O str) and 1090.63 cm^{-1} (C–O strong)[19]. Tween 20 also depicted characteristic bands at 3498.68 cm^{-1} (–OH, strong), 2868.32 cm^{-1} (–OH str very broad), 1734.64 cm^{-1} (5-membered ring), 1457.61 cm^{-1} ($–CH_3$). Similarly, the FTIR spectrum of tween 80 showed peaks at 3488.68 cm^{-1} (–OH strong), 2867.61 cm^{-1} (–OH strong), 1734.55 cm^{-1}, 1457.50 cm^{-1} ($–CH_3$), 1349.60 cm^{-1} (–OH in plane), and 1097.17 cm^{-1} (C–O–C acyclic) [20].

Cholesterol has key role in stabilizing the unilamellar or multilammellar bilayers [21]. The FTIR spectrum of cholesterol (Fig. 2) showed major peaks at 2931.41 cm^{-1} (Acetyl groups), 2866.83 cm^{-1} (symmetric $–CH_3$), 1770.20 cm^{-1} (vinyl group), and 1055.17 cm^{-1} (R–O strong) [22]. The characteristic peaks of individual components are shown in Table-1 and Fig. 2.

FTIR Spectra of Physical Mixtures (PMs)

Physical mixtures (PMs) of Diacerein with selected nonionic amphiphiles were prepared by simple mixing in equimolar ratios (Table-2) and were scanned as given in Fig. 3. The major peaks observed in spectra of PMS are listed in Table-3. The interaction between drug and carrier components may lead to detectable changes in FTIR scans. The characteristic m- substituted peak (1764 cm^{-1}) of Diacerein was found in all physical mixtures at 1735-1765 cm^{-1}. The findings of present study revealed that Diacerein showed similar peaks in spectra of physical mixtures as in individual spectrum of pure diacerein. The spectra of physical blends of Diacerein with selected nonionic amphiphiles did not depict identifiable shift of peaks, in particular of diacerein, indicating no interactions.

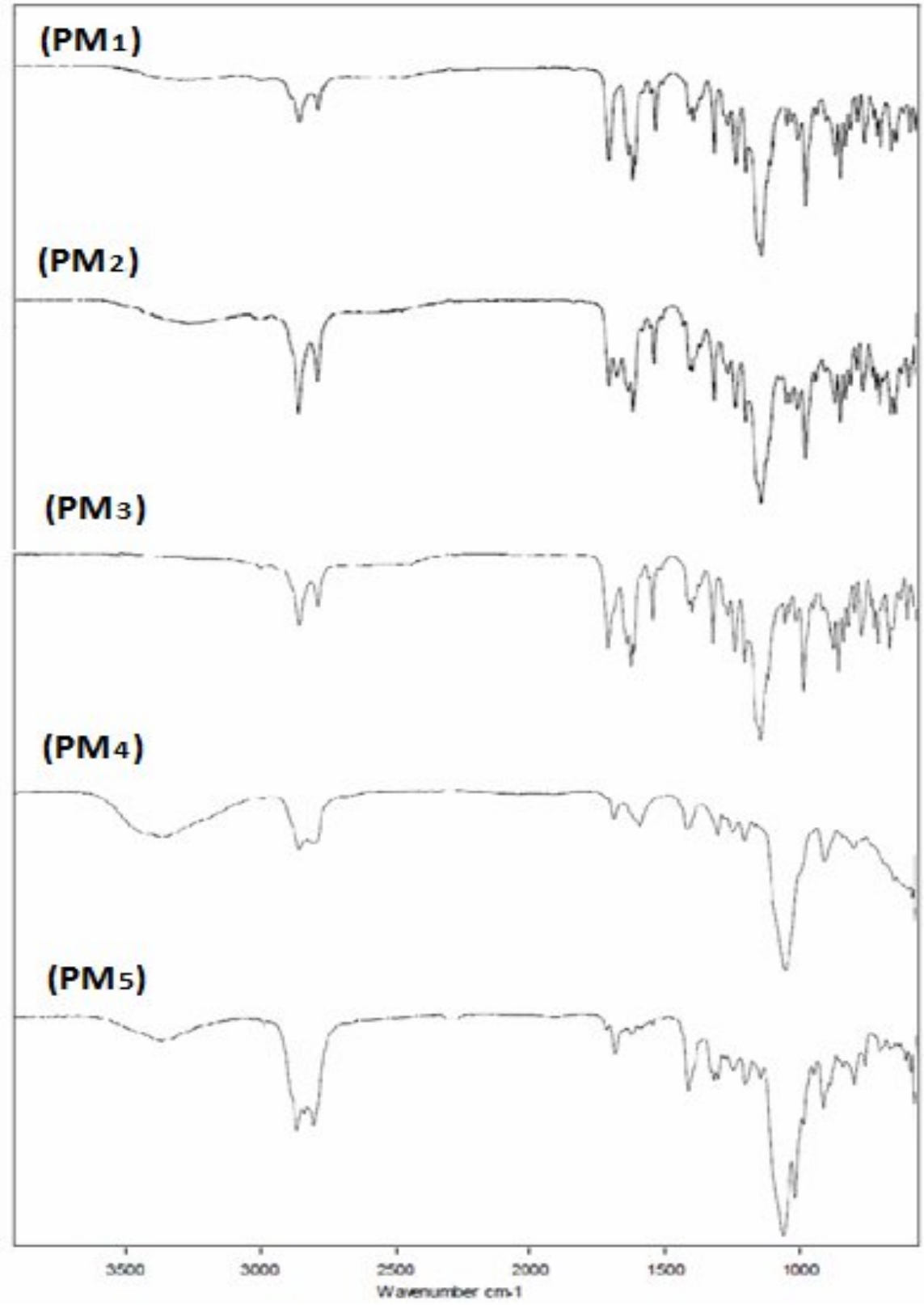

Fig. 3: FTIR Spectra of Physical Mixtures (PM_1, PM_2, PM_3, PM_4 and PM_5).

Table-1: Characteristic Peaks of Individual components.

Individual Components	Characteristic Peaks	Presence of Functional groups
Diacerein	3300 cm^{-1}, 3069.20 cm^{-1}, 1764 cm^{-1}, 760.43 cm^{-1}, 703.2 cm^{-1}	–COOH, C–H, C=O, m-substituted benzene, benzene
Span 60	2916.75 cm^{-1},1467.56 cm^{-1}, 1734.65 cm^{-1}, 1000to1200 cm^{-1}	–OH, $–CH_3$, Cyclic 5-membered ring, aliphatic chain
Span 80	2412.63 cm^{-1}, 2923 cm^{-1},1740 cm^{-1}, 1463.49 cm^{-1}	Aromatic ring, –OH, 5-membered ring, $–CH_3$
Span 85	2923.18 cm^{-1},1743.05 cm^{-1}, 1643.44 cm^{-1}, 1377.42 cm^{-1}	Double bond CH_2, Ketone with 5-membered cyclic ring, scissoring double bond CH_2, $–CH_3$).
Tween 20	3498.68 cm^{-1},1734.64 cm^{-1}, 1457.61 cm^{-1},	–OH group, 5 membered ring, $–CH_3$.
Tween 80	3488.68 cm^{-1},1457.50 cm^{-1}, 1097.17 cm^{-1}	–OH group, $–CH_3$,C–O–C Acyclic
Cholesterol	2931.41 cm^{-1},2866.83 cm^{-1},1770.20 cm^{-1}	Acetyl groups, symmetric $–CH_3$, Vinyl group

Table-2: Pure Ingredients and the composition of Physical Mixtures, and Niosomal formulations.

Codes	Ratio	Description of components
A	1	Drug Diacerein
B	1	Cholesterol
C	1	Span 60
D	1	Span 80
E	1	Span 85
F	1	Tween 20
G	1	Tween 80
PM_1	1:1:1	Diacerein + Cholesterol + Span 60
PM_2	1:1:1	Diacerein + Cholesterol + Span 80
PM_3	1:1:1	Diacerein + Cholesterol + Span 85
PM_4	1:1:1	Diacerein + Cholesterol + Tween 20
PM_5	1:1:1	Diacerein + Cholesterol + Tween 80
F_{SP60}	1:1:1	Diacerein + Cholesterol + Span 60
F_{SP80}	1:1:1	Diacerein + Cholesterol + Span 80
F_{SP85}	1:1:1	Diacerein + Cholesterol + Span 85
F_{Tw20}	1:1:1	Diacerein + Cholesterol + Tween 20
F_{Tw80}	1:1:1	Diacerein + Cholesterol + Tween 80

Table-3: Characteristic Peaks of Physical Mixtures (PM_1—PM_5).

Physical Mixtures	Characteristic Peaks	Presence of Functional Groups
PM_1	2920.32 cm^{-1},2850.21 cm^{-1}, 970.56 cm^{-1}, 1765.3756 cm^{-1}	–OH groups, –CH out of plane, vinyl ring
PM_2	2922.91 cm^{-1},2853.05 cm^{-1}, 1765.19 cm^{-1}, 760.44 cm^{-1}	–OH groups, –OH group, vinyl, substituted benzene
PM_3	3300 cm^{-1},2853.11 cm^{-1},760.33 cm^{-1}, 1189.76 cm^{-1}, 703.12 cm^{-1},	–COOH, –OH group, C–O group, substituted benzene
PM_4	2921.34 cm^{-1},1735.16 cm^{-1}, 1638.34 cm^{-1}, 947.31 cm^{-1}	–OH strong, C–O group, C=C group, –OH out of plane,
PM_5	3439.45 cm^{-1},2928.76 cm^{-1}, 1735.03 cm^{-1}, 741.18 cm^{-1},	–COOH, –OH group,C=O Substituted benzene

Table-4: Characteristic Peaks of Niosomal Formulations (F_{SP60}—F_{Tw80}).

Formulations	Characteristic Peaks	Presence of Functional Groups
F_{SP60}	3362.78 cm^{-1},2900 cm^{-1},1637.92 cm^{-1}, 760 cm^{-1}	–OH, C–H, C=C, disubstituted benzene
F_{SP80}	3375.27 cm^{-1}, 1635.93 cm^{-1}, 735 cm^{-1}-770 cm^{-1}	–COOH, C=C, disubstituted benzene
F_{SP85}	3379.85 cm^{-1}, 1634.89 cm^{-1}, 735cm^{-1}-770 cm^{-1}	–COOH, C=C, disubstituted benzene
F_{TW20}	3357.47 cm^{-1},1638.63 cm^{-1}, 735 cm^{-1}-770 cm^{-1}	–COOH, C=C, disubstituted benzene.
F_{TW80}	3355.60 cm^{-1}, 1637.97 cm^{-1}, 735 cm^{-1}-770 cm^{-1}	–COOH, C=C, disubstituted benzene.

FTIR Spectra of Niosomal Formulations

Niosomal formulations (Table-2) were prepared by thin film hydration method from equal molar ratios of nonionic surfactants and Diacerein. FTIR spectra of niosomal formulations (Fig. 4) showed the remarkable attenuation of IR bands of drug and nonionic surfactants with significant reduction in the intensity of peaks. The major peaks with corresponding functional groups are summarized in Table-4. The characteristic peak of carboxylic group (3300cm^{-1}) of Diacerein was most visible in all niosomal formulations at 3355-3379 cm^{-1}. Most of the peaks were found to be diffused indicating formation of niosomes. Moreover, major peaks of Diacerein were smooth indicating strong physical interaction between drug and surfactants. No additional peaks or shifting of peaks were observed in PMs and niosomal formulations indicating no chemical interaction between drug and nonionic surfactants [23] and the Diacerein was present in intact form and available for biological action.

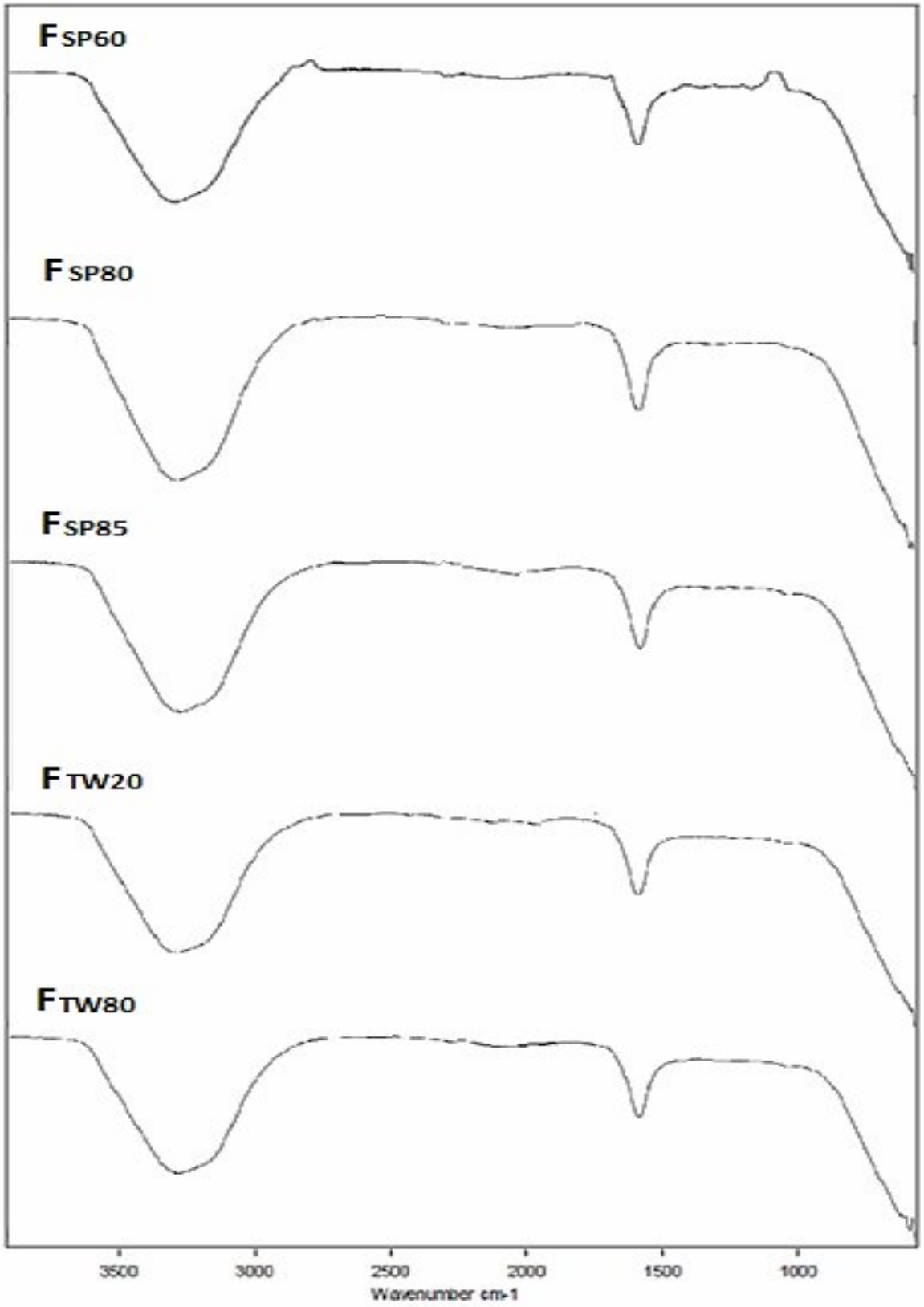

Fig. 4: FTIR Spectra of Niosomal Formulations (FSP60, FSP80, FSP85, FTW20 and FTW80).

Conclusion

ATR-FTIR analysis was successfully employed for identification of individual components, physical mixtures and the niosomal formulations. The results confirmed the absence of chemical incompatibilities/interactions between Diacerein and nonionic amphiphiles including span 60, Span 80, Span 85, Tween 20, and Tween 80. Therefore, it is concluded that Diacerein can be encapsulated into the niosomal vesicles with the aforesaid nonionic surfactants.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this work.

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