Compatibility analysis of bergapten with different pharmaceutical excipients used in nanostructured lipid carriers

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Abstract: In pharmaceuticals sciences, Fourier-transform infrared spectroscopy (FTIR) is a very useful technique to measure the compatibility and interaction between ingredients, therefore in the current study, compatibility of bergapten with different excipients was analyzed via FTIR. Nanostructured lipid carriers (NLCs) are the second generation of lipid nanocarriers and very useful for the drug delivery systems. Nanoparticles (NPs) were prepared by a nanotemplate engineering technique and scanning of pure drug, individual ingredients and, physical mixtures of different ingredients was carried out. The characteristic peak of the carboxylic groups of bergapten is shifted from 3088.1 cm^{-1} to 3399.3 cm^{-1} due to formulation development and it confirmed that it was properly incorporated into the formulation. Other peaks of the drug were also present in formulation with minor shortening/broadening of peaks. The resulted peaks of IR spectra depicted that the ingredients used in the formulation had no considerable interaction and were found compatible with each other.

Keywords: Fourier-transform infrared spectroscopy (FTIR), bergapten, physical mixture, nanostructured lipid carriers (nlcs), compatibility.

INTRODUCTION

A lot of analytical strategies have been used for the identification and qualitative/quantitative analysis of different compounds in plants, derived food products, and cosmetics. Previously, thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and gas chromatography (GC) have proven to be an irreplaceable tool for phytochemical profiling and for the definition of furanocoumarin content in plant samples. For identification and qualitative profiling of new compounds, the most frequently applied detection techniques are medium and high resolution mass spectrometry (MS, MS/MS, and HRMS), while for quantitative targeted purposes, other detection systems have proved to be useful, such as ultraviolet (UV), diode array (DAD) and fluorescence detection (FLD) (Bruni R, Barreca *et al.,* 2019).

Fourier-transform infrared spectroscopy is one of the widely accepted analytical techniques available for identification or confirmation of complex formation or new compound development. Samples in any state e.g. liquids, pastes, powders, and films can be analyzed by using this technique (Stuart 2005). Moreover, compatibility of ingredients and substances like alcohol, drugs, chemicals, and blood can also be checked through this technique (Theophanides 2012). Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) is a very beneficial tool to interpret structural variations of these compounds (Ślósarczyk *et al.,* 2005). The characteristics occurring IR spectra peaks of different compounds provide an ability to identify specific functional groups in pharmaceuticals dosage forms. Similarly, the possible relationship between IR absorbance and concentration facilitates the quantitative estimation of individual components of a formulation (Ahuja and Scypinski 2010, Khan *et al.,* 2015).

Bergapten, a linear furanocoumarins (psoralens) is obtained from *Cnidium manner* (Chinese medicinal herb) (Li and Chen 2004). The plants also contain coumarins, chromones, essential oils, terpenoids and glycosides (Yang *et al.,* 2003). Moreover, bergapten (5-methoxypsoralen (5-MOP) can also be obtained from bergamot oil (Averbeck *et al.,* 1990). This drug has inadequate water solubility and high lipophilic character which make it an ideal candidate for NLC encapsulation (Fang *et al.,* 2008). In the plasma of food, bergapten has C_{max} 37-144 $ng.ml^{-1}$ (Ehrsson *et al.,* 1994). Moreover, it is well known antimicrobial, anticancer, anti-inflammatory (Bose *et al.,* 2011), analgesic (Chen *et al.,* 1995), and osteoporotic (Zhang *et al.,* 2007).

Breast cancer is a leading type of cancer that is due to its invading potentials in developing countries with time

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(Carol *et al.,* 2014). Different medicinal products from natural origin can be modified by structural modification or by synthesis with other compounds to achieve desired therapeutic goals (Gordaliza 2007). Therefore, bergapten is one of the drugs having pronounced programmed metabolic activity against breast cancer cells (Santoro *et al.,* 2016). The structure of bergapten is given in fig. 1.

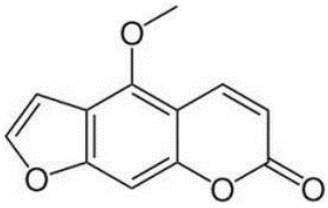

Fig. 1: Chemical structure of bergapten (4-methoxyfuro[3,2-g] chromen-7-one)

In the present work, FTIR analysis of pure bergapten, excipients i.e. Span 60, Tween 80, salicylic acid, migloyl and PEG 400, physical mixtures and formulation (NLC) was conducted. After recording FTIR spectra, the compatibility of ingredients and interaction between them was examined.

MATERIALS AND METHODS

Bergapten was imported from Chemfaces, Wuhan, China. Stearic acid, miglyol, sorbitan monopalmitate (Tween 80), sorbitan monopalmitate (Span 60), polyethylene glycol 400 (PEG 400) were purchased from Sigma Aldrich, Schnelldorf, Germany. All the chemicals were of analytical grade.

Development of nanostructured lipid carriers (NLCs)

Nanoparticles (NPs) were prepared by a nanotemplate engineering technique previously used by Kim *et al.* (2012) with slight modifications (Lu *et al.,* 2009, Kim *et al.,* 2012). Briefly, the mixture of drug, stearic acid, miglyol, Tween 80, Span 60 and PEG 400 (1:2:1:3:0.6:6 by weight) were melted in the water bath at 70°C. Pre-heated water for injection app. 10 mL was added to the melted mixture dropwise and clear nanoemulsions were formed after stirring (magnetic stirrer) at 70°C for 40 mins. Nanoemulsions were cooled down at 4°C to solidify the lipid core, leading to the formulation of lipid nanoparticles. Prepared NPs were then filtered through a 0.22 µm syringe filter to remove any large particles and unentrapped drug. NPs were lyophilized at -55°C at a vacuum of 1×10^{-4} mbar using freeze-dryer. Lyophilized NPs were reconstituted with water for injection or phosphate-buffered saline (pH 7.4) as per needed before use for further testing (Qureshi *et al.,* 2017). Physical mixtures of drug and the other ingredients were prepared by stirring with gentle heating for 10 to 15 mins. until the homogeneous mixture was achieved.

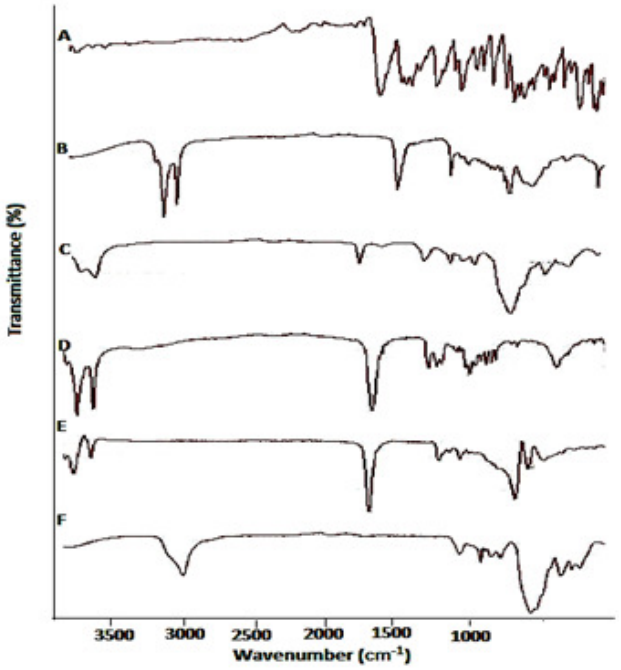

Fig. 2: FTIR spectra of bergapten (A), Span 60 (B), Tween 80 (C), Salicylic Acid (D), Migloyl (E), and PEG 400 (F).

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectroscopic studies

IR spectra of individual bergapten, migloyl, PEG400, Tween 80, Span 60 and salicylic acid were recorded along with the physical mixture of all these ingredients. The physical mixtures of bergapten + migloyl, bergapten + Tween 80, bergapten + Span 60, bergapten + stearic acid, bergapten + PEG 400, bergapten + Span 60 + Tween 80 + stearic acid, bergapten + migloyl + PEG 400, stearic acid + miglyol + Tween 80 + Span 60 + PEG 400, bergapten + stearic acid + miglyol + Tween 80 + Span 60 + PEG 400 and formulation (NLCs) were taken by scanning in wavenumber range of 4000-650 cm^{-1}.

RESULTS

ATR-FTIR spectroscopic method is the most applied technique to confirm any sort of interaction and compatibility of ingredients in physical/chemical and pharmaceutical study. So, it is very helpful to develop any sort of formulation and to characterize it well (Madni, *et al.,* 2017). The present work was designed to evaluate any interaction or incompatibility between the ingredients.

FTIR spectrum of bergapten

FTIR spectrum of bergapten depicts the characteristic peaks of carboxylic acid (O-H), alkane (C-H), aldehyde (C=O), phenol (O-H) and aliphatic ether (C-O) at 3088.1 cm^{-1}, 2981.4 cm^{-1}, 1720.2 cm^{-1}, 1351.2 cm^{-1} and 1120.1 cm^{-1} respectively. The spectrum of pure bergapten is not reported until now.

Table 1: FTIR spectra peaks of individual components

Peaks	Appearance	Groups	Class of compounds
Bergapten			
3088.1 cm^{-1}	Strong, broad	O-H (S)	Carboxylic acid
2981.4 cm^{-1}	Medium	C-H (S)	Alkane
1720.2 cm^{-1}	Strong	C=O (S)	Aldehyde
1351.2 cm^{-1}	Medium	O-H (B)	Phenol
1120.1 cm^{-1}	Strong	C-O (S)	Aliphatic ether
Span 60			
3388.3 cm^{-1}	Strong, broad	O-H (S)	Alcohol
2916.6 cm^{-1}	Weak broad	O-H (S)	Alcohol
2849.5 cm^{-1}	Medium	C-H (S)	Alkane
1735.1 cm^{-1}	Strong	C=O (S)	Ester aldehyde, lactone
1466.7 cm^{-1}	Medium	C-H (B)	Alkane
1172.2 cm^{-1}	Strong	C-O (S)	Ester, tertiary alcohol
721.2 cm^{-1}	Strong	C=C (B)	Alkene
Salicylic acid			
2914.8 cm^{-1}	Strong, broad	O-H (S)	Carboxylic acid
2847.7 cm^{-1}	Medium	C-H (S)	Alkane
1697.8 cm^{-1}	Strong	C=O (S)	Conjugated aldehyde, conjugated acid
1293.4 cm^{-1}	Strong	C-O (S)	Aromatic ester
933.7 cm^{-1}	Broad	C=C (B)	Alkene
721.2 cm^{-1}	Strong	C=C (B)	Alkene
Tween 80			
2857.0 cm^{-1}	Medium	C-H (S)	Alkane
1735.1 cm^{-1}	Strong	C=O(S)	Ester, aldehyde, lactone
1458.3 cm^{-1}	Medium	C-H (B)	Alkane
1246.8 cm^{-1}	Strong	C-O (S)	Alkyl aryl ether
1094.0 cm^{-1}	Strong	C-O (S)	Aliphatic ether, secondary alcohol
944.9 cm^{-1}	Medium	C=C (B)	Alkene
Migloyl			
2924.1 cm^{-1}	Weak, broad	O-H (S)	Alcohol
2853.3 cm^{-1}	Medium	C-H (S)	Alkane
1740.7 cm^{-1}	Strong	C=O (S)	Ester, aldehyde, lactone
1155.5 cm^{-1}	Strong	C-O (S)	Tertiary alcohol
1101.4 cm^{-1}	Strong	C-O (S)	Aliphatic ether
PEG 400			
3455.2 cm^{-1}	Strong , broad	O-H (S)	Alcohol
2864.5 cm^{-1}	Medium	C-H (S)	Alkane
1457.4 cm^{-1}	Medium	C-H (B)	Alkane
1349.3 cm^{-1}	Medium	O-H (B)	Alcohol
1094.0 cm^{-1}	Strong	C-O (S)	Aliphatic ether, secondary alcohol
939.6 cm^{-1}	Medium	C=C (B)	Alkene

S = Stretching, B = Bending

FTIR spectra of excipients

FTIR spectrum of Span 60 showed the peaks at 3388.3 cm^{-1}, 2916.6 cm^{-1}, 2849.5 cm^{-1}, 1735.1 cm^{-1}, 1466.7 cm^{-1}, 1172.2 cm^{-1}, 721.2 cm^{-1} with groups of O-H stretching (alcohol), O-H stretching (alcohol), C-H stretching (alkene), C=O stretching (ester, aldehyde, lactone), C-H bending (alkene), C-O stretching (ester, tertiary alcohol), C=C bending (alkene), respectively. The present work showing the FTIR peaks are quite similar to the previous work of Behera *et al.* (2012) and Khan *et al.* (2015).

FTIR spectra of salicylic acid indicated carboxylic acid, alkane, conjugated aldehyde and conjugated acid, aromatic ester and alkene showing the peaks at 2914.8 cm^{-1} (O-H stretching), 2847.7 cm^{-1} (C-H stretching), 1697.8 cm^{-1} (C=O stretching), 1293.4 cm^{-1} (C-O stretching), 933.7 cm^{-1} and 721.2 cm^{-1} (C=C bending), respectively. These result have resemblance to earlier research work shown by Ibrahim *et al.* (2005). Overall IR spectra of all ingredients is provided in fig. 2.

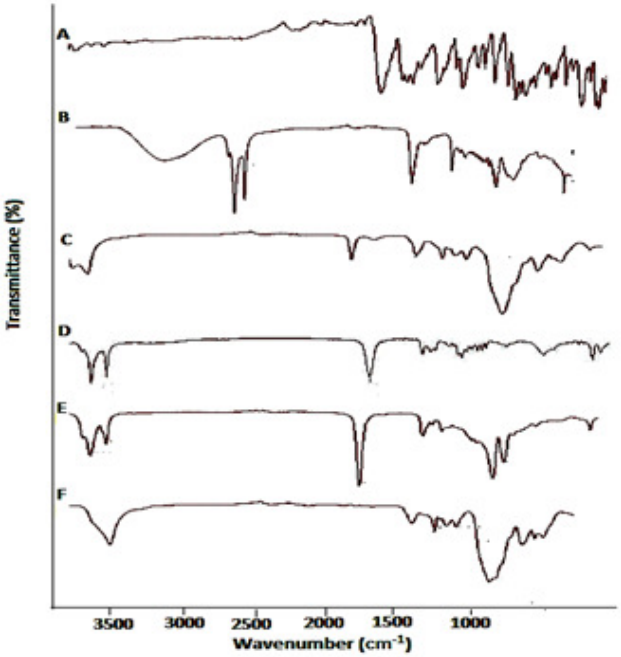

Fig. 3: FTIR spectra of (A) bergapten and physical mixture of bergapten with (B) Span 60, (C) Tween 80, (D) Salicylic acid, (E) Migloyl, and (F) PEG 400.

IR spectra of Tween 80 showed that C-H stretching (alkane) at 2857.0 cm^{-1}, C=O stretching (ester, aldehyde, lactone) at 1735.1 cm^{-1}, C-H bending (alkane) at 1458.3 cm^{-1}, C-O stretching (alkyl aryl ether) at 1246.8 cm^{-1}, C-O stretching (aliphatic ether, secondary alcohol) at 1094.0 cm^{-1}, C=C bending (alkene) at 944.9 cm^{-1}. These IR spectra peaks are also found compatible with previous results shown by Liu *et al.* (2015). Further details of all FTIR spectra peaks is shown in table 1.

Characteristic peaks of migloyl were present at 2924.1 cm^{-1}, 2853.3 cm^{-1}, 1740.7 cm^{-1}, 1155.5 cm^{-1} and 1101.4 cm^{-1} with the class of alcohol (O-H), alkane (C-H), ester, aldehyde, lactone (C=O), tertiary alcohol and aliphatic ether (C-O), respectively. The above peaks are quite the same as previous research results of Butstrean *et al.* (2014).

Furthermore, PEG 400 have peaks of 3455.2 cm^{-1} strong, broad O-H stretching alcohol, 2864.5 cm^{-1} medium C-H stretching alkane, 1457.4 cm^{-1} medium C-H bending alkane, 1349.3 cm^{-1} medium O-H bending alcohol, 1094 cm^{-1} strong C-O stretching aliphatic ether, secondary alcohol, 939.6 cm^{-1} medium C=C bending alkene and had similar to previous research work shown by Tunc et al. (2008).

FTIR spectra of physical mixture

The physical mixture was also scanned and IR spectra Span 60, Tween 80, salicylic acid with bergapten narrate the peaks at 2857.0 cm^{-1} strong, broad O-H stretching carboxylic acid present in bergapten and salicylic acid. 1735.1 cm^{-1} strong C=O stretching ester, aldehydewere recorded as depicted in Fig. 3. Spectrum of bergapten with Span 60 showed the appearance of strong, broad peaks at 3375.1 cm^{-1} which is O-H stretching (alcohol/carboxylic), 1735.1 cm^{-1} C=O stretching (ester, aldehyde, lactone) and 1176.0 cm^{-1}, C-O stretching (ester, tertiary alcohol group) present in both ingredients and 721.2 cm^{-1}, C=C bending (alkene) was the Span 60 peak. The medium peaks at 2916.6 cm^{-1} and 2849.5 cm^{-1} showed C-H stretching (alkane) and peak at 1466.7 cm^{-1} reflected C-H bending (alkane).

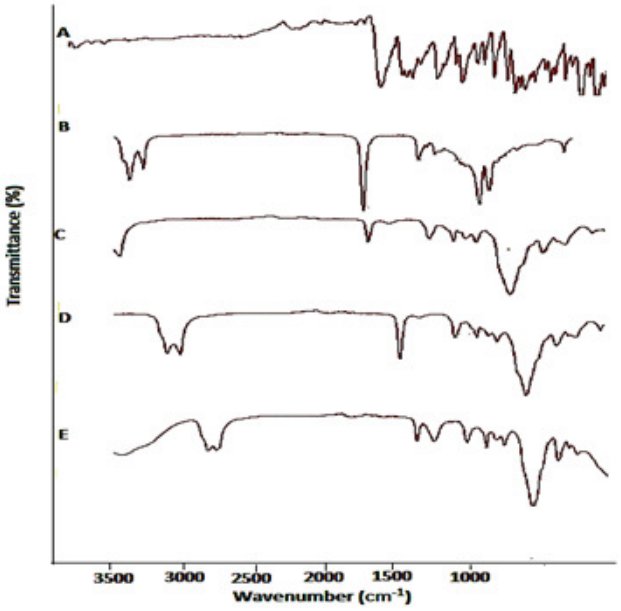

Fig. 4: Physical mixtures and NLCs spectra with comparison of drug (A) bergapten, (B) Migloyl + bergapten + PEG 400, (C) Span 60 + bergapten + Tween 80 + salicylic acid, (D) mixture of all the ingredients, and (E) nanostructured lipid carriers (NLCs).

In bergapten and Tween 80 mixture, the characteristic peaks of carboxylic acid (O-H stretching) at 2857.0 cm^{-1} was of bergapten, ester, aldehyde, lactone (C=O stretching) at 1735.1 cm^{-1} peak of both ingredients. Alkyl aryl ether (C-O stretching) at 1246.8 cm^{-1} was of Tween 80. Aliphatic ether, secondary alcohol (C-O stretching) at 1094.0 cm^{-1} peaks of both ingredient, alkene (C=C bending) at 944.9 cm^{-1} was only of Tween 80.

Bergapten and migloyl mixture depict the peaks at 2924.1 cm^{-1} medium C-H stretching alkane, 2855.1 cm^{-1} medium C-H stretching alkane 1740.7 cm^{-1} strong C=O stretching ester, aldehyde, lactone, which were present both in bergapten and migloyl. 1153.6 cm^{-1} strong C-O stretching tertiary alcohol characteristically of miglyol, 1101.4 cm^{-1} strong C-O stretching aliphatic ether, secondary alcohol was of both ingredients and 725.0 cm^{-1}

Table 2: IR spectra peaks of physical mixtures and NLCs formulation

Peaks	Appearance	Groups	Class of compounds
Bergapten: Span 60			
3375.1 cm^{-1}	Strong, broad	O-H (S)	Alcohol
2916.6 cm^{-1}	Medium	C-H (S)	Alkane
2849.5 cm^{-1}	Medium	C-H (S)	Alkane
1735.1 cm^{-1}	Strong	C=O (S)	Ester, aldehyde, lactone
1466.7 cm^{-1}	Medium	C-H (B)	Alkane
1176.0 cm^{-1}	Strong	C-O (S)	Ester, tertiary alcohol
721.2 cm^{-1}	Strong	C=C (B)	Alkene
Bergapten: Tween 80			
2857.0 cm^{-1}	Strong, broad	O-H (S)	Carboxylic acid
1735.1 cm^{-1}	Strong	C=O (S)	Ester, aldehyde, lactone
1246.8 cm^{-1}	Strong	C-O (S)	Alkyl aryl ether
1094.0 cm^{-1}	Strong	C-O (S)	Aliphatic ether, secondary alcohol
944.9 cm^{-1}	Medium	C=C (B)	Alkene
Bergapten: Salicylic acid			
2919.2 cm^{-1}	Strong, broad	O-H (S)	Carboxylic acid
2851.8cm^{-1}	Medium	C-H (S)	Alkane
1699.1 cm^{-1}	Strong	C=O (S)	Conjugated aldehyde, Conjugated acid
721.23 cm^{-1}	Strong	C=C (B)	Alkene
Bergapten: Migloyl			
2924.1 cm^{-1}	Medium	C-H (S)	Alkane
2855.1 cm^{-1}	Medium	C-H (S)	Alkane
1740.7 cm^{-1}	Strong	C=O (S)	Ester, aldehyde, lactone
1153.6 cm^{-1}	Strong	C-O (S)	Tertiary alcohol
1101.4 cm^{-1}	Strong	C-O (S)	Aliphatic ether, secondary alcohol
725.0 cm^{-1}	Medium	C=C (B)	Alkene
Bergapten: PEG 400			
2864.5 cm^{-1}	Medium	C-H (S)	Alkane
1349.3 cm^{-1}	Medium	O-H (B)	Alcohol
1094.0 cm^{-1}	Strong	C-O (S)	Aliphatic ether, secondary alcohol
941.2 cm^{-1}	Medium	C=C (B)	Alkene
Bergapten: Migloyl: PEG 400			
2924.1 cm^{-1}	Medium	C-H (S)	Alkane
2855.1 cm^{-1}	Medium	C-H (S)	Alkane
1740.7 cm^{-1}	Strong	C=O (S)	Ester, aldehyde, lactone
1459.3 cm^{-1}	Medium	C-H (B)	Alkane
1377.3 cm^{-1}	Medium	O-H (B)	Phenol
1151.7 cm^{-1}	Strong	C-O (S)	Tertiary alcohol
1101.4 cm^{-1}	Strong	C-O (S)	Aliphatic ether, secondary alcohol
723.1 cm^{-1}	Medium	C=C (B)	Alkene
Bergapten: Span 60: Tween 80: Salicylic acid			
2857.0 cm^{-1}	Strong, broad	O-H (S)	Carboxylic acid
1735.1 cm^{-1}	Strong	C=O (S)	Ester, aldehyde, lactone
1094.0 cm^{-1}	Strong	C-O (S)	Aliphatic ether, secondary alcohol
944.9 cm^{-1}	Medium	C=C (B)	Alkene
Bergapten: Span 60: Tween 80: Salicylic acid: Migloyl: PEG 400			
2922.2 cm^{-1}	Medium	C-H (S)	Alkane
2853.1 cm^{-1}	Strong, broad	O-H (S)	Carboxylic acid
1740.7 cm^{-1}	Strong	C=O (S)	Ester, aldehyde, lactone
1458.3 cm^{-1}	Medium	C-H (B)	Alkane
1097.7 cm^{-1}	Strong	C-O (S)	Aliphatic ether, secondary alcohol
946.7 cm^{-1}	Medium	C=C (B)	Alkene

Continue…

Nanostructure lipid carriers (NLCs)			
3399.3 cm^{-1}	Strong, broad	O-H (S)	Carboxylic acid
2920.4 cm^{-1}	Medium	C-H (S)	Alkane
1742.5 cm^{-1}	Strong	C=O (S)	Ester, aldehyde, lactone
1349.3 cm^{-1}	Medium	O-H (B)	Alcohol
1090.2 cm^{-1}	Strong	C-O (S)	Aliphatic ether, secondary alcohol
944.9 cm^{-1}	Medium	C=C (B)	Alkene

S = Stretching, B = Bending

strong C=O stretching ester, aldehyde, lactone, was depicted in all ingredients of mixture. 1094.0 cm^{-1} strong C-O stretching aliphatic ether was the part of bergapten and Tween 80, secondary alcohol and 944.9 cm^{-1} medium C=C bending alkene was shown in salicylic acid and Tween 80.

Bergapten, Span 60, Tween 80, salicylic acid, migloyl and PEG 400 physical mixture depicted the peaks at 2922.2 cm^{-1} medium C-H stretching (alkane group) is major one which is present in all ingredients, 2853.1 cm^{-1} strong, broad O-H stretching (carboxylic acid group) is the present in bergapten and salicylic acid, 1740.7 cm^{-1} strong C=O stretching (ester, aldehyde, lactone groups) was shown in all ingredients except PEG 400, 1458.3 cm^{-1} medium C-H bending (alkane group) was the member of Span 60, Tween 80 and PEG 400, 1097.7 cm^{-1} strong C-O stretching (aliphatic ether, secondary alcohol groups) was in bergapten, PEG 400 and Tween 80 and 946.7 cm^{-1} medium C=C bending (alkene group) was in salicylic acid, Tween 80 and PEG 400. So all ingredients characteristics peaks were available in this mixture. Further detail of all these mixture is available in Table 2.

FTIR spectrum of formulation (NLCs)

In case of NLCs spectrum, the different peaks were seen in Fig. 4 like at 3399.3 cm^{-1} O-H stretching (carboxylic acid), 2920.4 cm^{-1} C-H stretching (alkane), 1742.5 cm^{-1} C=O stretching (ester, aldehyde, lactone), 1349.3 cm^{-1} O-H bending (alcohol), 1090.2 cm^{-1} C-O stretching (aliphatic ether, secondary alcohol) and 944.9 cm^{-1} C=C bending alkene.

DISCUSSION

ATR-FTIR spectroscopic method is the most applied technique to confirm any sort of interaction and compatibility of ingredients in physical/chemical and pharmaceutical study. So, it is very helpful to develop any sort of formulation and to characterize (Madni *et al.,* 2017). IR spectra peaks of excipients shown in formulation with no shifting indicate that drug has no interaction with the excipients and are quite compatible with excipients. Moreover, spectrum of pure bergapten is not reported until now. The characteristic peak of carboxylic group of bergapten is visible but it is shifted from 3088.1 cm^{-1} to 3399.3 cm^{-1} due to formulation development and it means that it was properly incorporated into the developed formulation (Sütő *et al.,* 2016). Other peaks of drug were also present in formulation with minor shortening/broadening of peaks. The low intensity peaks shown broadness due to the other ingredients. This study shows that chemical interactions that could alter the structure of the drug does not occurs and chemical structure of the bergapten is likely to be unaffected in the presence of excipients.

CONCLUSION

ATR-FTIR spectroscopy is well-known technique used for compatibility and physical/chemical interaction studies of drug and inactive ingredients. Therefore, it is very helpful to analyze every type of pharmaceutical formulations. The nanotemplate engineering technique was used for preparation of NLCs. The drug (bergapten) used in formulation is also a promising candidate for many therapies. In this study, the NLCs of bergapten were analyzed by FTIR and findings were found compatible as peaks of IR spectra reflected that the ingredients used in the formulation had no considerable interactions with each other. For the future prospect, there are a lot of analysis techniques suggested such as HPLC, LC-MS and salting-out liquid-liquid extraction.

ACKNOWLEDGEMENT

Authors acknowledge Muhammad Mubbashir Khan (Quality Control Manager), Briell Pharmaceuticals (Pvt.) Ltd, Lahore for his skillful assistance in recording FTIR.

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