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RESEARCH ARTICLE

PHARMACEUTICAL ANALYSIS

INTERACTION STUDY OF NSAIDS WITH COW'S GHEE AND ITS FATTY ACIDS BY NMR SPECTROSCOPY

Corresponding Author

DR.B.ANILREDDY

Maheshwara college of Pharmacy, Patancheru, Hyderabad.

ABSTRACT

The possibility of interaction between NSAIDs namely; diclofenac sodium and acetaminophen with cow's ghee, oleic acid and palmitic acid has been investigated by ^{1}H-NMR spectroscopic technique, due to its capability to identify the protons involved in interaction, with precision and accuracy. Interaction can be easily identified by observation of selective line broadening or chemical shift displacements of ^{1}H-NMR signals. Acetaminophen was found sufficiently stable in presence of cow's ghee and fatty acids, as no detectable change was noticed in ^{1}H-NMR pattern of binary mixture. Significant changes were observed in the binary mixtures of diclofenac sodium as shown by deviation in chemical shift as well as broadening of signals, suggesting possible complexation between diclofenac sodium and cow's ghee or fatty acids.

KEY WORDS

Diclofenac sodium; Acetaminophen; Cow's ghee; Fatty acids; ^{1}H-NMR.

INTRODUCTION

Fats and fatty acids are the building block components of most of the lipids, which constitutes biomembranes. They are active in providing fluidity and flexibility to the membrane and thereby assisting transportation of materials across it[1]. Fats and fatty acids have been explored in pharmaceutical research for modulation of drug release from different dosage forms.

Cow's ghee (ghee) is an isolated fat of milk, obtained via fermentation of milk using strains of *Lactobacillus* and subsequently heat clarified to remove moisture[2]. Besides being used in ayurvedic formulations, ghee is also used in conjunction with food in daily Indian diets. Along with other substances, cow's ghee contains numerous saturated and unsaturated fatty acids. Among these fatty acids, palmitic acid, a 16:0 saturated fatty acid, constitutes 29.95% (Fig. 1), while oleic acid (Fig. 2), which is 18: 1 monounsaturated acid with a double bond between 9-10 carbon atoms, is present to the extent of 27.42%[3].

There are several reports on the influence of esters of fatty acids and fatty alcohols on in- vitro release of diclofenac sodium and acetaminophen. However no such work has been reported on ghee, which is known to be a pool of bioactive compounds[2], consisting of several saturated and unsaturated fatty acids alongwith some vitamins, minerals, carotinoids etc. Consequently an understanding of the mechanism involved in interaction, if any, between the drugs namely diclofenac sodium or acetaminophen with ghee, palmitic or oleic acid at molecular level using ^{1}H-NMR has remained unexplored. Such an investigation will be of great importance, because of the ability of this technique to identify the protons involved in interaction, if any, with more precision and accuracy.

Diclofenac sodium (Fig. 3), is {2-[(2,6-dichlorophenyl) amino] benzene acetic acid sodium salt}, possesses structural characteristics of both the arylalkanoic acid and the anthranilic acid class of anti-inflammatory agents and displays anti-inflammatory, analgesic, and antipyretic properties. Structure of diclofenac includes a phenyl acetic group, a secondary amino group, and a dichlorophenyl ring (chlorine atoms at ortho position)[4].

Acetaminophen, (N-acetyl-p-aminophenol), is one of the most effective antipyretic-analgesic but an ineffective anti-inflammatory drug (Fig. 4). It is official in many pharmacopoeias, and available in large number of official and proprietary products alone and in combination with other analgesics[5].

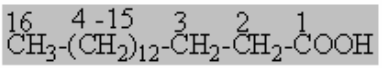

Fig. 1
Palmitic acid.

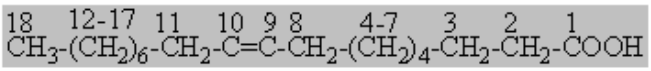

Fig. 2
Oleic acid.

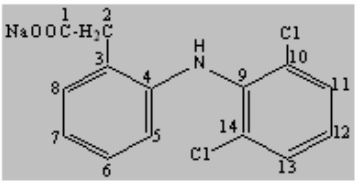

Fig. 3
Diclofenac sodium.

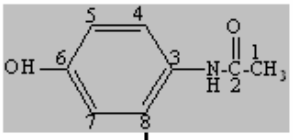

Fig. 4.
Acetaminophen.

EXPERIMENTAL

Materials

Diclofenac sodium and acetaminophen were obtained from Sun Pharmaceutical Industries Ltd., Mumbai, and Aristo Pharmaceuticals Ltd., Raisen, M.P., respectively as gift samples. Cow's ghee was purchased from Go-Vigyan Anusandhan Kendra, Kanpur, U.P. Palmitic acid and oleic acid was purchased from Loba Chemie, Mumbai and Merck, Schuchardt, Germany respectively. All other materials used were of A.R. Grade.

Method

Preparation of Sample

Fusion admixture of diclofenac sodium, acetaminophen with ghee and palmitic acid were prepared by melting the ghee and palmitic acid (2g) in a beaker kept over a water bath maintained at 65-70°C. To the molten ghee or palmitic acid, an equivalent amount of diclofenac sodium or acetaminophen were added, and uniformly dispersed by continuous stirring to prepare 1:1 (w/w) binary mixture. The 1:1 w/w ratio was selected to maximize the likelihood of observing any interaction[6]. The fused mixture was homogenized and allowed to cool slowly to room temperature with stirring. The binary mixture was stored in amber-colored glass bottles for 3months.

Weighed amount (2g) of diclofenac sodium or acetaminophen were incorporated into the equal amount of oleic acid with continuous stirring and stored in amber colored glass bottle for 3months.

Nuclear Magnetic Resonance Spectroscopy

^{1}H-NMR spectra were recorded in the solvent (CD_3OD: $CDCl_3$ 3:1, v/v), using tetramethylsilane (TMS) as an internal standard. The solutions of the samples were equilibrated in the probe for approximately 5min before each run. High-resolution ^{1}H-NMR spectra were obtained at room temperature using Bruker DRX-300 spectrometer operating at 300 MHz.

RESULTS AND DISCUSSION

The investigation of interaction between the two substances relies on the observation of selective line broadening and/or chemical shift displacements of ^{1}H-NMR spectral signals of a substance bonded with other[7]. Table 1, records ^{1}H-NMR data of the samples under investigation.

Table 1

Chemical shifts (δ) of different protons of drug and fatty acids.

H/ H'	(δ)ppm				($\Delta\delta = \delta_2 - \delta_1$)[11]ppm					
	DS[1]	AAP[2]	PA[3]	OA[4]	DS-PA[5]	DS-OA[6]	DS-CG[7]	AAP-PA[8]	AAP-OA[9]	AAP-CG[10]
1 H		2.08						0.00	0.00	0.00
4, 8 H		6.77, 6.74						0.00, 0.00	0.00	0.00,0.00
5 H	6.39				+0.01	+0.10	+0.05			
5, 7 H		7.33, 7.30						0.00, 0.00	-0.02	-0.02
6 H	6.98				+0.02	-0.02	-0.03			
7 H	6.84				+0.02	+0.04	+0.01			
8 H	7.22				0.00	0.00	-0.01			
11 H	7.39				+0.01	-0.05	-0.05			
12 H	6.98				+0.05	+0.06	0.00			
13 H	7.36				+0.01	-0.05	-0.05			
1 H'				11.40						
2 H'			2.284	2.31	-0.01	-0.05		0.00	-0.01	
3 H'			1.596	1.59	+0.01	0.00		0.00	0.00	
4 H'			1.312		-0.01			0.00		
5 H'			0.914		0.00			0.00		
(4-8) (11-17) H'				1.26, 1.31		0.00			0.00	
18 H'				0.88		0.00			0.00	
8,11 H'				2.03		0.00			0.00	
9,10 H'				5.30		+0.03			+0.02	

Diclofenac Sodium[1], Acetaminophen[2], Palmitic acid[3], Oleic acid[4], Diclofenac & palmitic acid[5], Diclofenac & oleic acid[6], Diclofenac & ghee[7], Acetaminophen & palmitic acid[8], Acetaminophen & oleic acid[9], Acetaminophen & ghee[10].

($\Delta\delta = \delta_2 - \delta_1$)[11], δ_2 ,chemical shift of binary mixture and δ_1 of drug/ fatty acid.

Cow's ghee is a complex mixture of different fatty acids along with amino acids, peptides, salts, vitamins etc. Other than palmitic and oleic acids the other major saturated fatty acids in ghee include myristic, stearic, lauric, butyric, capric, caprylic and unsaturated fatty acids like linoleic, linolenic, vaccenic and arachidonic acids[2]. At this stage it is difficult to propose any single chemical structure for ghee, hence the effect of ghee on NMR spectrum of drug was considered as an index of interaction between ghee and the drug.

In Fig. 5, the ^{1}H-NMR spectra of ghee, oleic and palmitic acids have been presented. An upward shift of 0.02ppm was noticed in the position of 5th and 7th proton of acetaminophen in acetaminophen -ghee mixture (Fig. 6 A). In case of binary mixture of acetaminophen with oleic acid, an upward shift of 0.02ppm in the position of 5th and 7th proton of acetaminophen was noticed (Fig. 6 B). In case of 9th and 10th proton of oleic acid, a downward shift of 0.02ppm was observed. No change in chemical shifts was observed in the binary mixture of acetaminophen with palmitic acid, ruling out any possibility of interaction (Fig. 6 C). In spite of having hydroxyl as well as amino group attached to the phenyl ring in acetaminophen, ghee, palmitic or oleic acid hardly showed any significant change in the chemical shift, suggesting the stability of acetaminophen in presence of ghee, palmitic or oleic acid. These results further confirms the outcome of previous two studies[8-9].

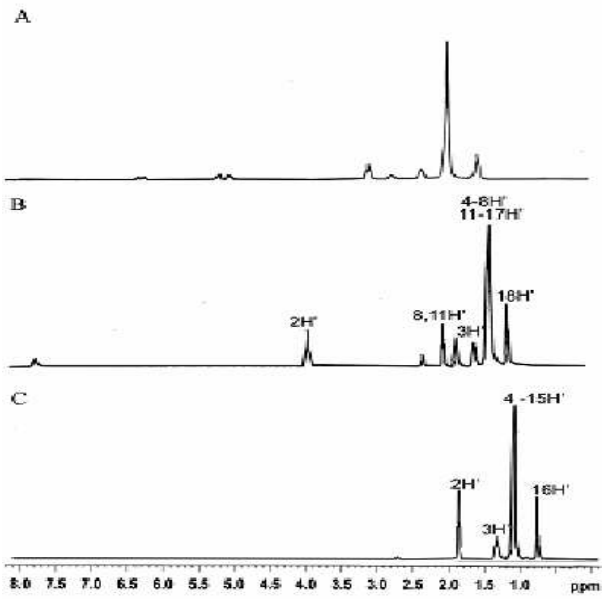

Fig. 5
^{1}H-NMR spectra of ghee (A), oleic acid (B), palmitic acid (C).

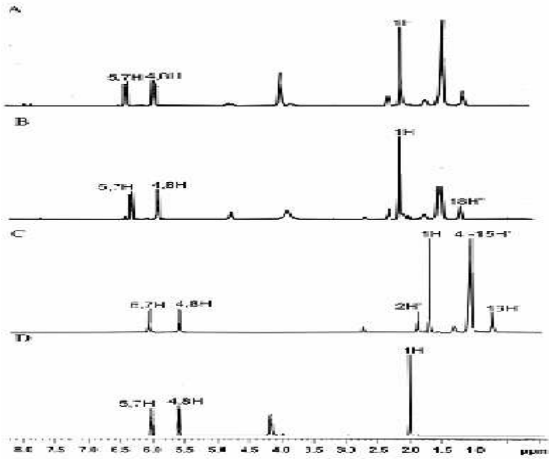

Fig. 6

[1]H-NMR spectra of binary mixture of acetaminophen with ghee (A), with oleic acid (B), with palmitic acid (C), acetaminophen (D).

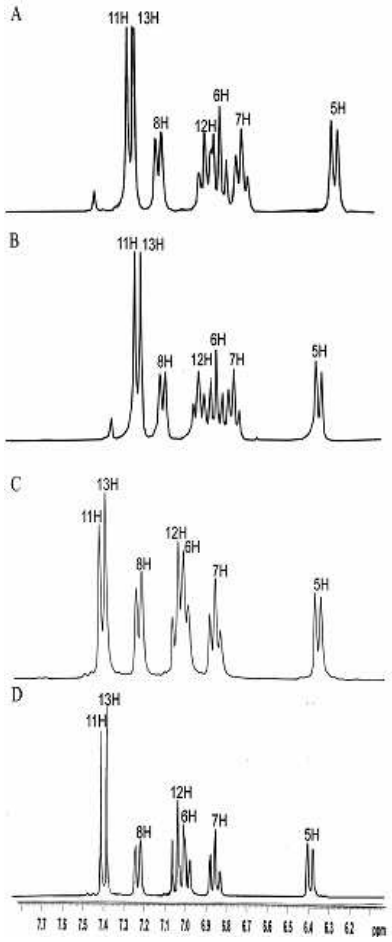

Fig. 7

[1]H-NMR spectra of binary mixture of diclofenac with ghee (A), with oleic acid (B), with palmitic acid (C), diclofenac (D).

The binary mixture of diclofenac sodium with ghee (Fig. 7A) showed more pronounced changes in chemical shift of different protons of diclofenac sodium than acetaminophen binary mixture with ghee, palmitic or oleic acid. Interaction is clearly evident in the binary mixture of diclofenac sodium with oleic acid because of difference in chemical shift values ranging from –0.05 to +0.06ppm (Fig. 7B). In the binary mixture of diclofenac sodium with palmitic acid (Fig. 7C), except for a few peaks, the position of remaining peaks were changed in respect to the position of different protons of diclofenac sodium. In all binary mixture of diclofenac, both aromatic rings appear to be involved in the interaction.

Because of hydrogen bonding, due to the ionic resonating structure, carboxylic acid exists as dimer in the solid or liquid state. In diclofenac sodium also, there exists an intramolecular hydrogen bond between the carbonyl oxygen and hydrogen of the amino group. Protons directly bonded to oxygen or nitrogen atom are more prone to undergo complexation because they are exchangeable, capable of forming hydrogen bonding and subject to partial or complete decoupling by the electrical quadrupole moment of the ^{14}N nucleus[10].

The changes produced by ghee in the spectral pattern are not only because of presence of palmitic or oleic acid in ghee but may also be the cumulative effect produced by different fatty acids. Other substances present in ghee may also contribute to these changes. The presence of proton on the oxygen and nitrogen atom in drugs makes the system more complicated because of occurrence of signals over a wide range in case of hydrogen bonding.

In the light of these facts, it can be postulated that there exists a strong possibility of complexation between the diclofenac sodium and ghee, palmitic acid or oleic acid. Because of the complex chemical nature of ghee it is not possible for us to specify the fatty acid or any other substance present in ghee, which may be responsible for the changes in the chemical shift of protons in diclofenac, at least at the moment. It might be because of the cumulative effect of a few or all saturated and unsaturated fatty acids along with some other substances present in ghee, which might be taking part in interaction simultaneously.

CONCLUSION

No interaction was detected in binary mixtures of acetaminophen with cow's ghee, oleic acid and palmitic acid however; interaction was evident in binary mixtures of diclofenac sodium. This might be because of some sort of complexation. Identification of a complexing compound should prove interesting. However, the extent to which such complexation may be useful in pharmaceutical practice should be of further interest. It is known that complexation usually leads to sustenance of release of material involved. Slow or sustained release formulations are important in pharmaceutical practice, and complexation reaction revealed in the present study should be useful from this point of view at least for topical applications. Medicinal properties of cow's ghee are an additional advantage in such formulations.

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