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In Vitro Absorption Studies of Ibuprofen with Cholic and Deoxycholic Acid Conjugates

K. K. VISHWAKARMA D. V. KOHLI* AND R. K. UPPADHYAY
Department of Pharmaceutical Sciences
Dr. H. S. Gour Vishwavidyalya, Sagar (M. P.) 470 003.

Cholic acid and deoxycholic acid were conjugated with glutamic acid to prepare N-[3α, 7α, 12α trihydroxy 24 -oxocholan - 24 yl] glutamic acid and N-[3α, 12α - dihydroxy - 24 oxocholan-24 - yl] glutamic acid. Deoxycholic acid was conjugated with α - alanine to prepare N-[3α, 12α-dihydroxy 24-oxocholan-24-yl] α- alanine. The sodium salt of cholic acid and deoxycholic acid conjugates were then prepared and evaluated for surface activity and emulsifying properties. The effect of these compounds on *in vitro* absorption of ibuprofen was also investigated. All the biosurfactants were found to enhance *in vitro* absorption of ibuprofen.

BILE salts are well known biosurfactants. These are important to stimulate biliary secretion, cholesterol elimination[1], neutralization of acid chyme,, absorption of lipid soluble substances[2] and vitamin A[3]. The ability of bile salts to solublize steroid hormones[4], antibiotics[5], dyes[6], and steroidal anti-inflammatory drugs[7-8] has been reported. The bile salts used in all these studies which are found in human bile, include sodium cholate, sodium glycocholate, sodium taurocholate and sodium deoxycholate. No attempt has thus far been made to change the structure of the side chain of bile acids. In the present investigation, the carboxylic group present in cholanic acid was conjugated with α - alanine and glutamic acid. The effect of this change in the structure of the side chain on the *in vitro* absorption of ibuprofen was studied.

EXPERIMENTAL

The melting point were determined in open capillaries using Toshniwal melting point apparatus and are uncorrected. IR spectra of the biosurfactants were recorded in KBr using Perkin Elmer 157 spectrophotometer. In addition to characteristic peaks, the peak at 3310-3135 cm^{-1} >C=O stretching vibrations of secondary amide was noted, common in each of the synthesised compounds.

*Corresponding author

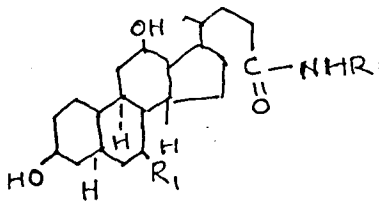
Synthesis of cholic and Deoxycholic acid conjugates

Sodium salt of N (3α, 7α, 12α, trihydroxy-24-oxocholan-24-yl) glutamic acid, N(3α, 12α dihydroxy-24-oxocholan-24-yl) glutamic acid and N(3α, 12α- dihydroxy-24-oxocholan-24yl) α-alanine were prepared adopting the method reported by Gautheir et al[9]. In brief, the triformyl cholic acid chloride and diformyl deoxycholic acid chloride was stirred with the corresponding amino acid in presence of 1M NaOH for 3 hours. The reaction mixture was allowed to stand for 24 hours, heated with gentle stirring at 60-65° for 30 minutes, cooled to 0°, precipitated with HCl and allowed to stand below 10° for 24 hours. The precipitate was then dissolved in hot 95 % C_2H_5OH poured into water and kept below 10° for 3-4 days, filtered and air dried. The product was then dissolved in sodium hydroxide solution (pH-8.3) and evaporated to dryness under reduced pressure to get the sodium salt of cholic and deoxycholic acid conjugates.

The following properties of the biosurfactants were evaluated.

Surface Tension : Surface tension of aqueous solution of biosurfactants (1% w/v) was determined using Fischer's surface tensiometer[10]. Results are shown in table - 1.

Table - 1: Surface Active Properties of Biosurfactants



Bio-surfactants	R1	R2	M.P.	Surface tension dynes/cm	Interfacial Tension dynes/cm	Emulsion Stability* at 30° 1 min	30 min.
CG	-CH-COONa CH_2 CH_2COONa	OH	137-138	44.00	28.00	2.50	5.80
TA	-CH-COONa CH_3	H	118.119	39.50	24.70	2.10	5.40
TG	-CH-COONa CH_2 CH_2COONa	H	147-148	37.90	23.10	1.80	6.70

The value of C, H and N were found within ± 0.4%

CG = Sodium salt of N-]3α, 7α 12α trihydroxy-24-oxocholan-24-yl] glutamic acid

TA = Sodium salt of N-[3α, 12α dihydroxyl-24-oxocholan-24-yl] alanine.

TG = Sodium salt of N-[3α, 12α dihydroxy-24-oxocholan-24-yl] glutamic acid.

Surface tension and interfacial tension expressed as dynes/cm at 30°.

Emulsion stability expressed as height of separated aqueous phase in (cm) after 1 and 30 minute intervals.

* A set of three readings were taken

Interfacial Tension : Interfacial tension of aqueous solution of the biosurfactants (0.1 % w/v) against liquid paraffin was determined by Traube's stalagmometer method[11]. The interfacial tension of liquid paraffin against water is taken as 35.5 dynes/cm. Results are given in table-1.

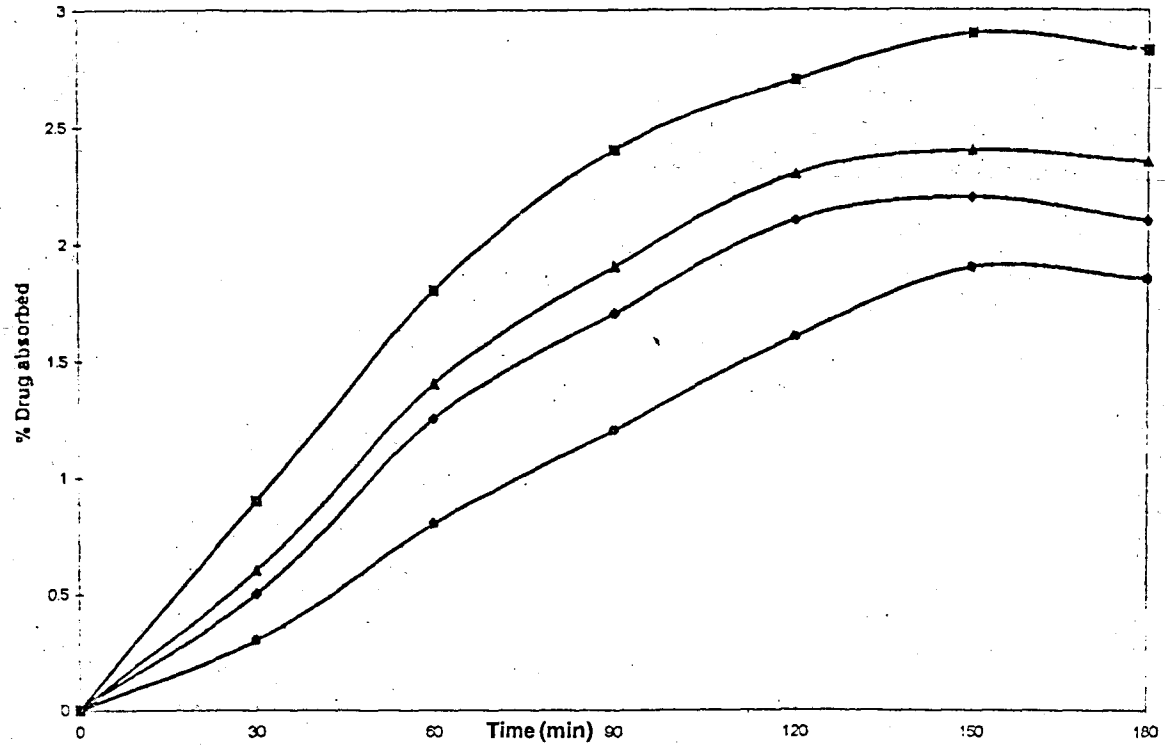
Emulsifying Properties: Creaming and settling are generally considered to be indices of emulsion stability even though they are not necessarily correlated with breaking of the emulsion[12]. The effects are frequently used as guidelines in the study of the stability of emulsion. The emulsion stability can be determined by noting the height of the separated phases. The aqueous solution of biosurfactants (20 ml, 0.5% w.v) was shaken with 15 ml of liquid paraffin in a 50 ml measuring cylinder (inner diameter 2.1 cm) for one hour in a shaker which imparted an average 110 longitudinal vibrations per minute, amplitude of each vibration being 11 cm. The height of the aqueous layer was noted at different time intervals during an hour. Results are given in the table-I.

***In Vitro* Absorption Studies:** The influence of the biosurfactants on the absorption[13,14] of ibuprofen was studied using living everted small intestine of rabbit. The method reported by Kohli *et al*[13] was followed for the *in vitro* absorption studies and the amount of ibuprofen absorbed into each loop was determined spectrophotometrically at 264 nm. Results are shown in figure 1.

RESULTS AND DISCUSSIONS

Biosurfactants form a class of compounds which have a structural similarity with the natural cholic salts. In natural process, the bile salts have been proven to enhance

Fig. 1: *In vitro* absorption studies of Ibuprofen



Percent Ibupforn absorbed through everted small intestinal loops of rabbit from plain (-O-) and emulisified ibuprofen with TA (-◆-), TG (-▲-) and CG (-□)-.

the uptake of hydrophobic dietary substances like triglycerides. The project was taken up with this basic aim.

All the synthesized compounds showed better surface active properties. All the three biosurfactants enhanced the absorption of ibuprofen (fig. 1). Sodium salt of N[3α, 7α, 12α trihydroxy-24 oxocholan-24-yl] glutamic acid was found to be a better surfactant in promoting absorption of ibuprofen through everted small intestine of rabbit than sodium salt of N[3α, 12α dihydroxy-24-oxocholan-24-yl] glutamic acid and sodium salt of N[3α, 12α dihydroxy-24-oxocholan-24-yl]α-alanine.

A large number of surface active agents are characterized by a molecular structure which has a clear cut polarity between hydrophilic and hydrophobic parts of the molecule. The common dihydroxy and trihydroxy bile salts, on the other hand, are steroids which possess a rigid cyclopentano phenanthrene nucleus, on one side of which are clustered hydroxyl groups and on the other the methyl groups. Protruding from one end of the steroid nucleus is a short aliphatic chain terminating in a strongly hydrophilic group. The molecule contains one hydrophobic side, one hydrophilic side and a short hydrophilic tail. Because of their molecular structure, bile salts do not bahave in a fashion analogous to ordinary aliphatic detergents. (Small et al., 1969)[16]. Lengthening of this hydrophilic tail by the use of amino acids leads to better hydrophilic lipophilic balance which is reflected in enhanced absorption of ibuprofen. The enhancement in the absorption of the drug may be due to the modification of permeability of biological membrane[17,18] besides the micellar solubilization or improved wettability of the drug in the presence of these biosurfactants.

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