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CHARACTERIZATION OF THE PRECIPITATES OF POORLY WATER SOLUBLE DRUGS AND DETERMINATION OF THE SOLUBILITIES IN HUMAN GASTRO-INTESTINAL FLUIDS BY MICROSOPY

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ABSTRACT

Gastrointestinal (GI) fluids were collected from 5 volunteers, and the pH of each fluid measured. Solutions of poorly water soluble sompounds in polyethylene glycol 400 (PEG-400) and absolute ethanol were introduced in measured aliqouts, to 2 mL portions of the GI fluids that were preheated to 37 C. The fluids were examined microscopically after each addition to determine the solubility of the drugs in those fluids and what type of precipitate would result. Melting temperatures of the compounds ranged from 40 C to 200 C. In all cases, the precipitate formed oily droplets in the GI fluids when the solubility limit was exceeded. Oily droplet formation was independent of molecular structure, melting temperature, and fluid pH. Compounds of low water solubility would be expected to form oily droplets in biological fluids, which would enhance absorption regardless of pH. GI fluid pH values were found to vary widely among individual samples from 1.49 to 7.08 (gastric) and 2.18 to 7.06 (intestinal).

INTRODUCTION

Oral dosages are the most common delivery route for drugs (1). Prior to penetration of the GI barrier, drugs are exposed by necessity to lumenal fluids. Gastric emptying, dissolution and decomposition are often rate limiting in the

sequence of absorptive events. Solubilized compounds are usually absorbed more rapidly than other dosage forms (1). Highly lipophilic drugs may be made more water soluble by salt formation, coadministration with surfactants, formation of prodrugs, or more recently by nanoparticulate formulations (1- 6). The latter approach uses the rationale that higher surface area, in conjunction with the proper surfactant, increases dissolution. With the solution dosage, poorly water soluble drugs may still have good bioavailability (1). The compounds are thought to precipitate out of solution in the GI fluids, and a rapid redissolution of the precipitates to occur (1, 4). Micelle formation is credited with the increased solubility of drugs in GI fluids, but the nature of the precipitates has not been reported. It is known that natural surfactants increase solubility, dissolution rates, absorption and bioavailability of solid dosages (7-13). Surface tension of gastric fluid also plays a role in dissolution kinetics (14).

An investigation was undertaken to determine the solubility of four poorly water soluble drugs in human GI fluids and the nature of the resulting precipitates. The compounds were chosen on the basis of a variety of melting points.

MATERIALS AND METHODS

Healthy paid volunteers, having no known history of gastrointestinal disorders or substance abuse, and free of prescribed medication for the preceeding month, were admitted to the Sterling Winthrop Pharmacology Study Unit at the Albany Medical Center Hospital in Albany, New York. The volunteers fasted overnight. Participants in this study are noted with an asterisk in Table 1. The remaining data have been collected from previous unpublished investigations but are noted here for informational purposes for the reader. All fluids were collected by approved clinical, protocol methods.

INTESTINAL FLUID COLLECTION

Cetacaine spray and viscous lidocaine were used to facilitate oral intubation of a triple lumen catheter. No other medications were administered to the subjects. Placement of the distal end of the catheter beyond the Ampulla of Vater was confirmed by fluoroscopy. After an initial collection of intestinal fluid (IF), Kinevac (cholecystekinin) was administered to simulate the fed condition

TABLE 1

Data on volunteers and gastric fluid (A) and intestinal fluid (B) collected at the Sterling Winthrop Pharmacology Study Unit at Albany Medical Center Hospital. Fluids used in this study are noted with an asterisk (*).

A.

SEX	AGE (yrs)	WEIGHT(lbs)	pH	
F*	38	140	7.08	*(a)
F*	64	135	2.76	
F	35	140	1.49	
F	30	140	1.88	
F	43	135	6.57	
M	44	198	2.08	
M	24	145	2.06	
F	36	170	2.11	
M	36	210	2.14	
F	44	150	2.17	
F	42	210	5.89	
M	50	155	1.71	
M	25	205	5.06	

B.

SEX	AGE(yrs)	WEIGHT(lbs)	pH fasted	pH fed
M*	26	166	7.06	2.18
M*	31	158	2.04	1.92
F*	43	133	6.69	6.56

*(a) pH adjusted to 1.95 with 0.1 N HCl.

by stimulation of the gall bladder. A second IF sample was then collected from each subject.

GASTRIC FLUID COLLECTION

Gastric fluid was obtained from volunteers using a standard clinical nasogastric tube. Placement of the tube was verified by auscultation. Kinevac was not administered.

PRECIPITATION AND SOLUBILITY DETERMINATIONS

The GI fluids were centrifuged to separate any solid matter. The pH of the supernatant was measured on a Beckman ϕ71 pH meter (Beckman Instruments, Inc., 4550 Norris Canyon Road, San Ramon, California). The supernatant was

heated to 37 C in 2 mL aliquots. Experimental compounds were obtained from Sterling Winthrop Pharmaceutical Research Division. Drug solutions were previously prepared in PEG-400 or absolute ethanol at concentrations of 1 and 10 μg/mL respectively. The drug solutions were added to the preheated (37 C) supernatants in 10 to 50 μL portions and examined on a glass slide with a Leitz Microscope (Upstate Technical Equipment, Syracuse, New York) at 320X using a long focal length objective and a Hot Stage apparatus (Mettler Instrument Corporation, Highstown, New Jersey) set to 37 C. The slide preparation was rechecked during the next 15 min for crystal formation. The procedure was repeated with water as a substitute for the GI fluids. Photomicrographs were taken with an Olympus OM-2S 35mm camera (Olympus, Crossways Park, Woodbury, New York).

RESULTS AND DISCUSSION

pH values of aspirated gastric fluids and those of gastric contents have been found to be in close agreement (15). The pH variation in Table 1 is unrelated to age, sex or weight. The additional data are presented for informational purposes only. These fluids were collected between 6:00 and 7:30 A.M. Previous studies have shown a circadian pattern of acidity, with a pH higher than 6 in the early morning hours between 4:00 and 7:30 A.M. (15-18). Since the accepted pH for gastric contents in the fasted state is approximately 2, the pH of the gastric fluid from one volunteer (pH 7.08) was adjusted to 1.95 prior to use (19).

Physical characteristics of the tested compounds are shown in Table 2. These properties were experimentally determined in the laboratory by Sterling Winthrop Inc. Low water solubility is the common factor. Needle crystals or amorphous precipitates formed immediately when the drug solutions came in contact with water at 37 C (Table 3). All crystals were between 2 to 6 μm long. The amorphous precipitates were less than 2 μm in diameter.

Table 4 shows the solubility of each compound in GI fluids. Solubility was based on the drug concentration at which oily droplets were seen (Fig. 1). The photomicrograph was taken at 640X to better visualize the droplets. The white droplets are beyond the field of focus. The oily droplet formation was identical for each compound. Excessive amounts of the drug solution were added to the GI

TABLE 2

Physical properties of four compounds tested for solubility in human gastrointestinal fluid.

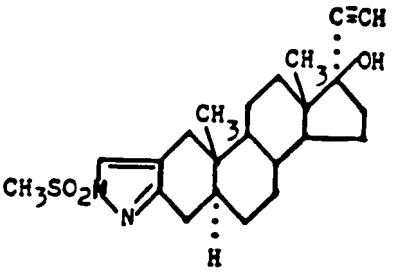
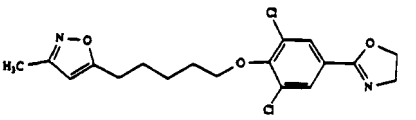
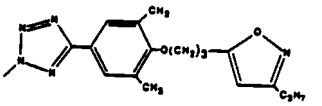
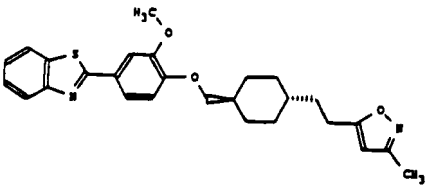
Compound	Structure	Molecular Weight	Solubility in Water (µg/mL)	Melting Temperature (Centigrade)
A		416.6	<8	200-202
B		383.3	<10	42-43
C		355.4	<2	63-65
D		462.6	<1	149-151

TABLE 3

Precipitate formation in water, resulting from ethanol or PEG-400 solutions of four poorly water soluble compounds added to water at 37 C.

	COMPOUND			
	A	B	C	D
Concentration (μg/mL)	<10	<50	<30	<50
Precipitate type and size (μm)	amorphous <2	crystals 2-6	crystals 2-6	crystals 2-6

TABLE 4

Approximate solubility concentrations of four compounds (μg/mL) in human gastric and intestinal fluids at 37 C, observed microscopically at 320X.

Fluid	Fluid pH	COMPOUND A	B	C	D
Gastric	2.8	150-190	180-220	70-100	40-60
Gastric	7.1*	160-200	170-220	70-100	50-70
Intestinal (fasted)	2.0	5-10	350-370	50-70	10-20
Intestinal (fed)	1.9	5-10	450-475	300-330	60-80
Intestinal (fasted)	6.7	5-10	210-230	40-60	20-30
Intestinal (fed)	6.6	5-10	250-280	250-270	60-80
Intestinal (fasted)	7.1	5-10	200-225	70-100	20-30
Intestinal (fed)	2.2	5-10	260-280	280-300	50-70

* pH adjusted to 1.95 with 0.1 N HCl.

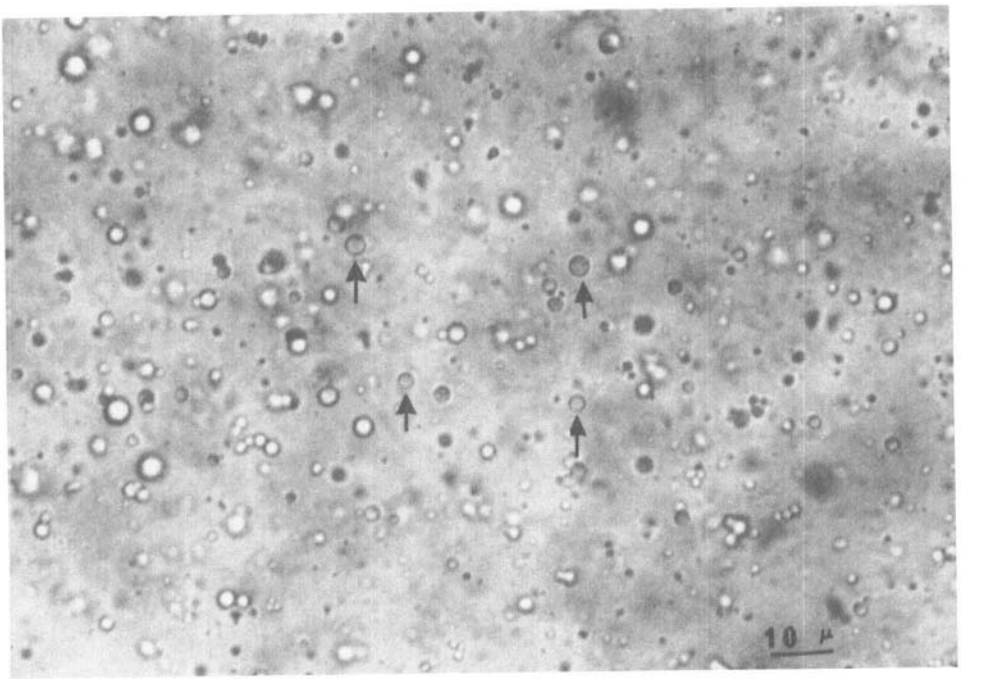


FIGURE 1

Photomicrograph taken at 640X, of compound A as oily droplets (indicated by arrows) in Human Intestinal Fluid, at a drug concentration of 30 μg/mL.

fluids in trial runs to make it possible to discern the drug oily droplets from ordinary fluid components. GI fluids, after centrifugation, have microscopic solid matter. The drug oily droplet formation differs from the GI fluid matter by its fluid movement and larger, uniform size of approximately 3 μm. The droplets did not coalesce, but some aggregation occurred. The oily droplets remained as stable formations for over 15 minutes. Similar results were reported by Serajuddin et al., using a water miscible organic solvent with a drug having low water solubility (4). In that study, the organic solution was added to water. Eventually crystals did form.

In this investigation, only compound A formed crystals: after 8 min in IF A, "fed" and fasted, and after 15 min in IF B, "fed" and fasted, on the hot stage. Compound A formed needles in IF C at room temperature after 10 min. Crystal

formation was probably aided by some liquid evaporation from the slide preparation. Crystals did not form in the 2 mL drug spiked IF. No crystals were seen in the gastric fluids, and no other compound formed crystals in the IF. In water, compounds B, C, and D formed crystals where A resulted in an amorphous precipitate (Table 3). This effect may be related to the melting temperatures of the compounds and rapid formation of the precipitate. There was a drug solubility difference between the gastric and intestinal fluids (Table 4). Compounds B, C, and D also had different solubilities in each of the intestinal fluids as well as in the "fed" and fasted state fluids from the same volunteer. Those differences are obviously unrelated to pH of the GI fluids or the melting temperatures of the compounds. Solvent concentration was not considered to play a role in the solubility or in the differences in solubility, since the solvent concentrations in the GI fluids ranged from 0.25 to 2.3%. PEG-400 and ethanol did not visually show additional precipitation of fluid components even when blank solvent was added to GI fluids at a 1:1 ratio. Any co-solvent effects of PEG-400 or ethanol at these concentrations would be negligible since the same drug solutions gave much lower solubility results in water (Table 4).

Micelle formation may account for the increased drug solubility and stabilization of the oily globules since the globules were visuallized at 320X. Micelles would not be visible by light microscopy. The types of micellar solubilization recognized are: (a) nonpolar solubilization where the drug is incorporated into the hydrocarbon center of the micelle, (b) polar-nonpolar solubilization, in which the drug is oriented in the palisade layer of the micelle in the same manner as the surfactant molecule, and (c) adsorption solubilization, where the compound is adsorbed onto the polar surface of the micelle (8, 20, 21). Micellar adsorption may have occurred along the oily globule surface, after the critical micelle concentration was exceeded, but this does not explain the oily droplet formation.

CONCLUSIONS

Drug solubility is many times greater in GI fluids than in water. This solubility differs in gastric and intestinal fluids, and in the fed versus the fasted state IF. Compounds of low water solubility would be expected to precipitate as oily droplets when delivered as a solubilized dosage. Melting temperature, molecular structure, and pH of the fluids are unrelated to the oily droplet

formation. The oily droplets would enhance absorption since the solubilized form of a drug generally maximizes bioavailability (1).

Since a circadian pattern of gastric acidity exists, there may be an optimum time for drug delivery based on ionization of the compound. Since the gastric pH flux is at least 5 units, IF pH's should be measured for 24 hrs to determine whether patterns of acidity exist for these fluids. The varied pH's of the fluids in Table1 indicates differences in the amount of acid compounds present.

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