Chewing Gum: A Novel Oral Dosage Form for Cocaine and Coca Leaf Extract

by

Sun Dong Yoo

A thesis presented to the University of Manitoba in partial fulfillment of the requirements for the degree of Master of Science in Faculty of Pharmacy

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CHEWING GUM: A NOVEL ORAL DOSAGE FORM FOR COCAINE
AND COCA LEAF EXTRACT

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ABSTRACT

In the present study a novel oral dosage form containing either cocaine or coca leaf extract in chewing gum was formulated and tested in vitro to be used for possible evaluation for the treatment of motion sickness during space flight.

The determination of cocaine potency of the several cocaine containing preparations was performed by a gas chromatographic (GC) procedure using flame ionization detection and the n-propyl ester of benzoylecgonine (n-PEBE) as internal standard.

The cocaine content of powdered leaves of Erythroxylum coca and Erythroxylum novogranatense var. truxillense was determined by extracting cocaine with ethanol, acid-base partitioning and quantification by GC.

An ethanolic extract of two types of coca leaf was prepared by extraction followed by percolation. GC fingerprinting was performed on these coca extracts, which served as a confirmatory aid in the standardization of the two coca leaf extracts.

Cocaine- and coca-dextrin were prepared by adsorbing cocaine hydrochloride or the residue of the ethanolic extract of powdered leaves of E. novogranatense var. truxillense onto dextrin. Chewing gum formulations containing co-
caine-free dextrin were first prepared and examined to
determine the gum composition offering optimum kneadability
and consistency. The final formulation used to prepare co-
caine- and coca-chewing gums consisted of 26% gum base, 13%
syrup, 13% water, 1% glycerin, and 47% cocaine- or coca-
dextrin. The cocaine content of chewing gums was determined
by an extraction process, acid-base partitioning followed by
GC quantification.

In vitro release studies were performed to characterize
and compare the release profiles of cocaine from cocaine-
and coca-chewing gums in both water and artificial saliva at
37°C. Of the total cocaine released, more than 80% was re-
leased from cocaine- and coca-chewing gums both in water and
artificial saliva in the first 20 min of the 80-min test
period. The release profiles of cocaine from all chewing gum
formulations studied in these media were reproducible.

The release data were also analyzed according to a
pharmacokinetic model. In terms of the rate and extent of
drug delivery as measured in the present in vitro release
study, the cocaine chewing gum is a more dependable, effi-
cient formulation than coca-chewing gum as an oral dosage
form of cocaine. An in vitro release study in man, however,
will be necessary to provide the definitive release profile
of cocaine from these chewing gum formulations.
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Chapter I
INTRODUCTION

1.1 ERYTHROXYLUM COCA

1.1.1 Pharmacognosy

Coca refers to several species and varieties of the genus Erythroxylum of the family Erythroxylaceae. The name "Erythroxylum" is derived from the two Greek words erythros (red) and xylon (wood) with the Latin ending -um, referring to the reddish wood of some species (1). The genus Erythroxylum includes as many as 250 species most of which are native to the American tropics.

Cultivated coca comes from the two closely related species of Erythroxylum coca Lamarck and Erythroxylum novogranatense (Morris) Hieronymus (2). Only these two species and varieties contain sufficient cocaine in their leaves to be used for either coca chewing or commercial extraction of coca alkaloids.

E. coca known as "Bolivian" or "Huanuco" coca is the most important commercial species of coca. This species is extensively cultivated in wet, tropical valleys on the eastern slopes of the Andes between the altitudes of 300 and 1800 meters (3). E. coca grows as a shrub or small tree at-
taining a height of one to two meters. The plant is recognized by the relatively large and thick, broadly elliptic, dark green leaves. They have a greater prominence of "lateral lines" which run parallel to each side of the midvein on the lower surface of the leaves.

A distinct form of E. coca is cultivated on a small scale in the western Amazon basin, in parts of Colombia, Peru, Ecuador, and Brazil. Plowman (4) has named this Amazonian coca as a new variety, Erythroxyllum coca var. ipadu Plowman. It differs from the typical Andean species in a number of morphological and chemical features. The leaves of the Amazonian coca are broadly elliptic and round at the apex. The branches are usually slender and quite erect. The parallel lines which are characteristic of E. coca are often faint or absent in the Amazonian variety. The Amazonian coca often has a larger and broader stigma than the Andean E. coca. The leaves of the Amazonian coca usually contain lower amounts of cocaine than those of Andean E. coca (4).

E. novogranatense, referred to as "Colombian coca", is the second most cultivated species of coca. Colombian coca is distinguished from E. coca by its smaller and thinner leaves. The leaves are brighter and more yellowish-green and are round at the apex giving the plant larger, bushier and more robust appearance. This species is cultivated in hot and seasonally dry areas of the Colombian mountains and on the Caribbean coast. Regarding alkaloid content, E. novogranatense is comparable to E. coca (5).
E. novogranatense var. truxillense (Rusby) Machado is known as "Peruvian" or "Trujillo" coca. This plant is similar to the Colombian coca in morphological and chemical features. Trujillo coca shows greater tolerance to the desert conditions found in coastal Peru. Trujillo coca is the variety used for the preparation of a coca-flavored beverage (Coca Cola®) because of the large amounts of methyl salicylate and other flavoring compounds it contains. This species may be distinguished from Colombian coca by its smaller and narrower but slightly thicker leaves with rich green color.

Besides cultivated species of the coca plant, some wild species of Erythroxylum are occasionally used for coca chewing. E. anquifurum and E. campestre were used as a medicine in Brazil (6). E. fimbriatum Peyr. and E. macrophyllum Cav. have been used as a substitute for E. coca var. ipadu in the region of Amazonian Peru (7). These species, however, contain negligible amounts of cocaine (8).

1.1.2 The Practice of Coca Chewing

Coca leaves have been a very important part of the cultural life of the South American Indians long before the Inca Empire was established. Coca leaves have been widely used as a medicine among native Indians in the Andes regions for at least several thousand years (9, 10).
During the time of the Incas the coca plant was regarded as a divine plant. In the Inca religion coca was believed to be a gift of Manco Capac, Royal Son of the Sun God. Its use was a privilege of the Inca aristocracy in official and religious ceremonies and for medical purposes (11). Following the Spanish conquest of the Incas, the use of coca spread to the lower classes including the mine workers and rural peasants.

At the present time coca chewing is a common practice in the Republic of Bolivia and Peru and also in the Amazon basin. In Bolivia and Peru coca chewing is especially widespread among the Quechuan Indians of the Altiplano (high plateau) and Puna (table land), and it is estimated that four million people are coqueros, or coca chewers (12).

Coca leaves are chewed by the natives as a mild stimulant. Coca chewing is believed to alleviate hunger and thirst, to help the natives work by reducing fatigue while augmenting endurance, and to promote a feeling of warmth in the user (13, 14). Coca leaves have also been widely used as a remedy for a variety of ailments such as indigestion, stomach ache, rheumatism, headache, external sores, and for the prevention of infirmity of the teeth and gums (15). Monge (16) has reported that there is a strong relationship between the prevalence of coca chewing and high altitude. Coca chewing is believed by the Indians to be helpful in maintaining their adaptation to the high altitude (13, 17).
Coca, because of its beneficial effect on respiration of people living at high altitude, has frequently been administered to relieve the alarming symptoms of nausea, dizziness, severe headache, or complete prostration in soroche or mountain sickness induced by the low oxygen content of the air and the low atmospheric pressure of the Andean highlands

The process of coca chewing, termed "chaccar" or "acullicar" in Peru and Bolivia, remains essentially the same now as it was in the times of Incas and is quite uniform throughout regions of coca use. The details of coca chewing are described in several articles (15, 19, 20).

Chucheros, or coca chewers, usually carry a small woven bag, chuspa, which contains a supply of coca leaves. Dried coca leaves are taken out of the chuspa, and the wad of leaves is then placed in the mouth. The leaves are moistened with saliva, chewed and turned around until they form a gum. The quid of softened leaves is held between the cheek and gums, and sucked with the tongue to extract the green juices. The leaves are not actually chewed but sucked allowing the juices to trickle into the stomach. During the chewing process some form of alkaline substance is always added to the quid of coca leaves. The alkaline substance used varies among regions of coca use. It may consist of bones, oyster shells or ashes of various plants and roots. The alkaline mixture known as llipta, tocra, or mambe is
taken from a calabash gourd known as an ishcupuru in Peru and a poporo in Colombia by means of a small moistened stick, and then added to the quid. The entire lump is kept in the mouth typically for about 45 minutes, being pushed by the tongue from one cheek to another while alkaline substance is added to it from time to time. The time the effects of a chew of coca continues is referred to as a cocada. Sometimes the cocada may last up to 2 or 3 hours.

The preparation of coca for use and the method of mastication in the Northwest Amazon basin, however, have been found to be different from those on the Andean highlands (4). In the former region the coca leaves known as ipadu are roasted on an oven until all are dry and crisp. They are ground into a fine green powder in a large wooden mortar with a pestle of hardwood. The ashes of burnt leaves of imbauba or varumo (various species of Pourouma and Cecropia) or other various plants are thoroughly mixed with the green powder. The fine sifted mixture is then put into the mouth, moistened and packed with the tongue between the cheek and gums. It is allowed to mix with saliva and most of the powder passes into the stomach.
1.2 COCA ALKALOIDS

1.2.1 Chemistry

The alkaloids found in a number of species of the genus *Erythroxylum* consist mainly of esters of ecgonine derivatives or hydroxytropanes. These alkaloids include cocaine, cinnamoylcocaine, pseudococaine, truxilline, tropacocaine, benzoyltropine, benzoylecgonine, ecgonine methyl ester, ecgonine, methyl ecgonidine, hygrine, hygroline and cuscoh-ygrine (21, 22). Chemical structures of cocaine and other alkaloids occurring in the leaves of the species of *Erythroxylum* are summarized in Fig. 1.

Besides these alkaloids, phenolic compounds, isoprenoids and some other minor constituents have also been found in coca leaves. Plowman (8) has reported that both varieties of *E. novogranatense* contain higher concentrations of essential oils consisting mainly of methyl salicylate than *E. coca*. Flavonoid ombuin-3-O-rutinoside has been found in abundance in both varieties of *E. novogranatense* whereas it is absent in *E. coca* (23). Mono-, sesqui-, di-, tri-terpenoids, tannins, fatty acids and wax alcohols have also been found as minor constituents in coca leaves.

Holmstedt (5) analyzed 13 species of *Erythroxylum* for cocaine content using a mass-fragmentographic technique. Cocaine was detected in significant quantity only in the cultivated species of *E. coca*, *E. novogranatense*, and *E. novo-
1) Cocaine
   \[ R'^1 = \text{CH}_3, \ R'^2 = \text{COC}_6\text{H}_5 \]

2) Cinnamoyl cocaine
   \[ R'^1 = \text{CH}_3, \ R'^2 = \text{COCH}=\text{CH}_6\text{H}_5 \]

3) Benzoylecgonine
   \[ R'^1 = \text{H}, \ R'^2 = \text{COC}_6\text{H}_5 \]

4) Ecgonine methyl ester
   \[ R'^1 = \text{CH}_3, \ R'^2 = \text{H} \]

5) Ecgonine
   \[ R'^1 = \text{H}, \ R'^2 = \text{H} \]

6) Cinnamoylecgonine
   \[ R'^1 = \text{H}, \ R'^2 = \text{COCH}=\text{CH}_6\text{H}_5 \]

7) Methylecgonidine
   \[ R'^1 = \text{CH}_3, \ \text{2,3 with no hydroxyl at C-3} \]

**Figure 1: Chemical structures of cocaine and other alkaloids occurring in the leaves of the species of Erythroxylum.**
granatense var. truxillense ranging from 0.13% to 0.76%. The wild species which are morphologically close to E. coca contained negligible amounts of cocaine. Aynilian (24) detected trace amounts of cocaine in wild species of the genus Erythroxylum.

Plowman (8) analyzed the dried leaves of 31 species and two varieties of the genus Erythroxylum for cocaine and found that the cultivated species contained varying amounts of cocaine (0.23 to 1.02%) depending on the leaf specimens and their extent of deterioration under different storage conditions. Small amounts of cocaine were detected in the leaves of 13 of the 29 wild species.

The chemical structure of cocaine was established in 1923 by the synthesis of cocaine by Willstätter et al. (25). The configuration of cocaine was determined more recently (26). Cocaine is benzoylmethylecgonine with the empirical formula C_{17}H_{21}NO_{4} and mol.wt. 303.4. Its entry in Chemical Abstracts is 3-((benzoyloxy)-8-azabicyclo-[3.2.1] octane-2-carboxylic acid methyl ester [1R-(exo-exo)]. Cocaine has a m.p. of 98°C and is slowly volatile above 90°C. One gram (1.0 g) of cocaine dissolves in 600 mL water, 0.7 mL chloroform, 3.5 mL ether and 6.5 mL ethanol (27). The aqueous solution is alkaline to litmus, has a slightly bitter taste and when applied to the tongue produces temporary anesthesia (27). Cocaine is a base with pKa of 8.6. It is most easily extracted at alkaline pH, but becomes unstable at el-
evated pH due to chemical hydrolysis (28). Cocaine is also hydrolyzed by serum cholinesterase. This hydrolysis can be inhibited by freezing (29) and by the addition of fluoride or cholinesterase inhibitors (30).

Cocaine hydrochloride which is the main form used in medicine is a salt with mol. wt. 339.81. One gram of the salt dissolves in 0.4 mL water, 3.2 mL cold alcohol, 2 mL hot alcohol and 12.5 mL chloroform, but is insoluble in ether or oils (31).

Econine methyl ester, benzoyleconine and econine are the major breakdown products of cocaine hydrolysis. Econine methyl ester may be obtained by refluxing a mixture of methanol and hydrochloric acid with econine (32). Econine methyl ester has a mol. wt. of 199.3 and exists as an oil at room temperature. Partial hydrolysis of cocaine by boiling in water produces methanol and benzoyleconine (33). Benzoyleconine has a mol. wt. of 289.3 and a m.p. of 195°C (dec). When boiled with dilute hydrochloric acid, benzoyleconine gives econine and benzoic acid. Hydrolysis of cocaine with dilute sulfuric acid also yields econine as well as benzoic acid and methanol (34). Econine is closely related to tropine differing from the latter only in having a CHCOOH group instead of CH₂ in the 2 position. Econine has a mol. wt. of 185.2, a m.p. of 205°C (dec) and a pKa of 11.11. Benzoyleconine and econine are very soluble in hot water, ethanol, acid and basic media, owing to their ampho-
teric nature. They are insoluble in ether, chloroform and other organic solvents.

1.2.2 Pharmacology

Cocaine is pharmacologically the most important constituent of the alkaloids of the genus *Erythroxylum*. It exerts actions on both the peripheral and central nervous (CNS) systems. It is classified as a local anesthetic and as a psychomotor stimulant (35). Cocaine has a very powerful anesthetic effect on the mucous membranes of the ear, nose and throat with temporary vasoconstriction.

Cocaine has two distinctive neuropharmacologic actions. First, it blocks the generation and conduction of sensory nerve impulses in nerve fibres by direct action on the cell membrane. Cocaine interferes with the process fundamental to the generation of the nerve action potential which causes a transient increase in the permeability of the axon membrane to positively charged sodium ions outside the cell. The mechanism whereby cocaine changes the axon membrane is not clearly understood, but it is thought that the drug may dissolve in the lipid matrix of the membrane and bind to the receptor sites within the sodium channel (36). The presence of the drug molecule at the receptor site interferes with the opening of the sodium channel, thus preventing the depolarization of the axon and blocking the nerve impulse (37). Secondly, cocaine inhibits the re-uptake of neurotransmitter
at sympathetic neurons, resulting in potentiation of the action of endogenous and exogenous catecholamines (38). These molecules tend to remain in the synaptic cleft where they can continue to stimulate the receptors in the dendrites of the adjacent nerve cell. Prevention of this uptake leads to increased levels of circulating catecolamines and sensitizes heart muscle, blood vessel and bronchioles to the effects of sympathetic stimulation, e.g., vasoconstriction, tachycardia, mydriasis and increased gastrointestinal motility (39, 40).

The action of cocaine on the medulla results in an initial increase in the respiratory rate followed by a decrease in the depth of respiration (41).

The vasomotor and vomiting centers may also be affected at high cocaine levels, resulting in emesis and a variety of cardiovascular effects. Small amounts of cocaine cause a slowing of the heart rate, but large quantities increase blood pressure (42).

Cocaine elevates body temperature by increasing muscular activity which increases heat production, and by vasoconstriction which diminishes heat loss.

Because of its stimulant effect on the CNS, cocaine has a potential for abuse. In animals, the administration of cocaine initially produces an elevation of mood, euphoria and perceptual changes (43). It has been reported that there is
a positive relationship between peak plasma concentrations of cocaine and its subjective and cardiovascular effects (44). The intense euphoria from cocaine, however, may be related to the rapidly increasing concentrations of cocaine at brain receptor sites but not to the peak concentrations in plasma (45). Higher doses or chronic use of cocaine causes degeneration of the CNS resulting in incoordinated movements, sleeplessness, hallucinations, delirium, psychosis, tremor and eventual convulsions (46, 47). There is no distinct withdrawal syndrome resulting from chronic cocaine use but continued craving for the drug, fatigue, lassitude, and depression has been reported (48).

Liver damage has been reported to occur notably in the mouse following chronic or acute administration of cocaine (49). The hepatotoxicity of cocaine appears in the form of infiltration, necrosis and marked increase in serum-glutamic oxaloacetic transaminase (SGOT) levels. It has been suggested that an active metabolite of cocaine, norcocaine nitroxide, might be responsible for the hepatotoxicity (50, 51). The mechanism of the bioactivation of cocaine to the more hepatotoxic metabolite is thought to consist of an initial dealkylation of cocaine to norcocaine via direct cytochrome P-450 oxidation or via sequential catalyzation with FAD-containing monooxygenase and cytochrome P-450, followed by the N-hydroxylation of norcocaine to N-hydroxynorcocaine by FAD-containing monooxygenase, and finally the oxidation of N-hydroxynorcocaine to norcocaine nitroxide (51).
The LD₅₀ of cocaine has been reported as 250 mg/kg s.c., 17.5 mg/kg i.v. in rats; 50 mg/kg s.c., 20 mg/kg i.v. in guinea-pigs; 126 mg/kg s.c., 17 mg/kg i.v. in rabbits; and 13 mg/kg i.v. in dogs (52, 53). Bedford et al. (54) reported that LD₅₀ of cocaine is 95.1 mg/kg in mice following IP injection. The LD₅₀ in man is approximately 500 mg after oral ingestion (55). The fatal dose of cocaine in man is thought to be 1.4 gm after oral ingestion, 750-800 mg by i.v. or s.c. injection or by inhalation (56). The upper limit for a safe dose is considered to be 200-300 mg for intranasal anesthesia (57). Fatalities, however, have been reported after as little as 30 mg of cocaine applied to the mucous membrane (58). The toxic blood level of cocaine has not been clearly established.

Norcocaine, like cocaine, possesses potent stimulant activity when it is administered to the rat by intracisternal or i.v. injection, while ecgonine and ecgonine methyl ester are inactive by both routes. Benzoyllecgonine and benzoylno-recgonine are active only by the intracisternal route (59). Norcocaine is several times more potent than cocaine as a local anesthetic (60). It also produces a greater elevation in the respiratory rate in monkeys than cocaine does (61).
1.2.3 Disposition

Cocaine is metabolized in humans and laboratory animals by the two distinct pathways of de-esterification and N-demethylation. Hydrolysis of the ester linkages of the cocaine molecule represents the major route of cocaine metabolism (62). Cocaine can be de-methylated and further oxidized to give norcocaine and related metabolites.

Nayak et al. (63) have reported the fate of [³H]-cocaine in acutely and chronically treated rats. When cocaine was injected intravenously into rats at a dose of 8 mg/kg, the peak plasma levels of cocaine in brain, plasma, and other tissues occurred within 0.25 h. The highest concentration of cocaine was observed in the spleen followed by the kidney, lungs, brain and testes. Liver and fat had lower concentrations when compared to other tissues. The initial peak levels of cocaine in plasma declined quickly as a result of tissue distribution, metabolism and some excretion. High lipid solubility of cocaine was considered to play an important role in its rapid entry into the brain. The approximate elimination half-life of cocaine in rat brain and plasma was found to be 0.4 and 0.3 h, respectively. No significant difference was found in plasma protein binding (mean value, 33.4% to 36.8%), and in peak levels of cocaine in brain and plasma after a 20 mg/kg s.c. injection in acutely and chronically treated groups of rats. Benzoylcegonine, benzoylnorecgonine, ecgonine and ecgonine methyl ester as well as un-
metabolized cocaine appeared in the urine following s.c. administration of a 20 mg/kg dose of [$^3$H]-cocaine (63). The excretion of free cocaine in urine amounted to only about 1 to 1.5% of the dose.

Norcocaine, benzoylcegonine and benzoynorecgonine have been shown to be metabolites of cocaine in the rat brain (64). In addition to these metabolites, evidence for the presence of phenolic and ring-hydroxylated metabolites has also been obtained (65). A very small amount of unchanged cocaine was excreted in the bile after a 5 mg/kg i.v. dose in rats (63). Evidence for glucuronide conjugation of cocaine and its metabolites has not been obtained, but the possibility of formation of conjugates with hydroxylated metabolites exists (66).

Rat liver microsomal enzymes have been found to convert cocaine to norcocaine in an *in vitro* system (67).

The known and hypothetical pathways for cocaine metabolism in the rat are outlined in Fig. 2.

Misra et al. (68) described the dispositional profile of cocaine in acutely and chronically treated dogs. The half-lives of cocaine in plasma and liver of dogs after a single i.v. dose of 5 mg/kg cocaine were 1.2 h and 2.2 h, and after chronic administration 1.1 h and 1.8 h, respectively. Norcocaine, benzoylcegonine, benzoynorecgonine and ecgonine were identified as metabolites of cocaine in the dog brain in
Figure 2: Known (→) and hypothetical (→→) metabolic pathways of cocaine in the rat.
both groups. The mean percent of cocaine excreted unchanged in urine and feces was 3.0 and 1.4, respectively. Norcocaine, benzylecgonine, benzoylecgonine, norecgonine, eegonine methyl ester and eegonine were identified as urinary metabolites of cocaine in both groups.

In man, Fish et al. (69) reported that 29 to 45% of an intramuscularly injected dose of cocaine was excreted in the urine as benzylecgonine. Wallace et al. (70) found benzylecgonine as a metabolite of cocaine as well as unchanged drug following the topical administration of cocaine hydrochloride to the nasal mucosa.

Van Dyke and co-workers measured cocaine plasma levels after intranasal and oral cocaine in humans (45, 71). A 10% solution of cocaine was applied to the nasal mucosa of surgical patients at a dose of 1.5 mg/kg. The plasma concentrations of cocaine increased rapidly for the first 15 to 20 min, peaked at 15 to 60 min, and then gradually decreased over the next 3 to 5 h. The half-life of cocaine was 2.5 h. The value, however, was regarded to represent a combination of absorption and elimination since cocaine remained on the nasal mucosa for 3 h due to the delayed absorption as a result of its vasoconstrictive action. In the other study (71), a 10% solution of cocaine hydrochloride and crystalline cocaine hydrochloride in a gelatine capsule were administered at a dose of 2 mg/kg to 4 subjects. After intranasal application of the solution peak concentrations occurred at
60 to 120 min, and then gradually decreased over the next 2 to 3 h. After oral administration of the capsules cocaine was not detected in the plasma until 30 min and then rapidly increased for the next 30 min. Peak plasma concentrations occurred at 50 to 90 min. The difference in mean plasma concentrations for the two routes of administration was not statistically significant. The peak plasma concentrations, however, were higher after oral cocaine than after intranasal cocaine at the same dosage.

Javaid et al. (44) administered 16 or 32 mg of cocaine intravenously, and 16, 64 or 96 mg of cocaine intranasally to ten volunteer subjects. Plasma concentrations of cocaine after intranasal doses increased rapidly for the first 20 to 30 min, peaked before 60 min, and then gradually decreased over the next hour. The half-life of cocaine after i.v. injection ranged between 16 to 87 min.

The primary site of cocaine metabolism in man was thought to be in the liver (48) occurring principally through hydrolysis, but some N-demethylation has been demonstrated in the liver (72). Recent research has suggested that serum cholinesterase plays a major role in metabolizing the drug (73). Plasma cholinesterase hydrolyzes cocaine by splitting one or both of the ester linkages (74).

The stability of cocaine in refrigerated blood samples (pH=7.4) spiked with cocaine was determined by Liu et al.
Seven percent (7%) of the cocaine was degraded to benzoylcegonine after 1 day, and 30% after 36 days. Benzoylcegonine was not further changed over the 36 days of the study period. Benzoylcegonine was considered to be formed non-enzymatically at physiological pH in man since neither serum nor liver esterases produce this compound from cocaine.

Inaba et al. (73) found eegonine methyl ester to be the major metabolite of cocaine after oral administration, accounting for 32 to 49% of all metabolites in urine. Stewart et al. (76) examined the action of liver and serum esterases on cocaine in vitro. Both the liver and serum were found to form eegonine methyl ester and benzoic acid enzymatically from cocaine.

Wilkinson et al. (77) studied the pharmacokinetics of the disposition of cocaine after oral and intranasal administration to healthy male subjects. Oral cocaine was administered as the hydrochloride salt in a dose of 2.0 mg/kg in a gelatin capsule. Oral cocaine disposition was described by a 1-compartment open model with first-order elimination and absorption with a lag time of 30 min. Intranasal cocaine kinetics were described by a 1-compartment open model with first-order elimination and absorption. The mean half-life after oral administration was 47 min, whereas the mean half-life after the intranasal route was 75 min and 73 min by solution and crystalline forms, respectively. The t-max was 64
min after oral administration, 58 min after intranasal administration of cocaine solution, and 35 min after intranasal application of crystalline cocaine. The relative bioavailability between the oral and intranasal routes was not significantly different. The fact that the difference in mean bioavailability between the two routes was not significant suggested that the liver accounts for a smaller proportion of cocaine metabolism than previously assumed and also indicates the apparent lack of hepatic first-pass effect.

Barnett et al. (78) studied cocaine pharmacokinetics in humans. A 100 or 200 mg dose of cocaine was administered to 4 subjects by i.v. infusion over a period of 1.5 to 3 min. For the 100 mg i.v. study the nonlinear regression analysis of the blood level data yielded a monoexponential function as the best fit, whereas for the 200 mg dose the results could be described by either a single exponential or a biexponential function. The biological half-life of cocaine ranged from 40 to 80 min and the total body distribution, \( V_b \), 1.2 to 1.9 L/kg, indicating that substantial binding to extravascular tissues occurs. Total plasma clearance values were 1.3 to 2.0 L/h per kg for the 100-mg studies and approximately one-half as large for the 200-mg studies. Dose dependent behavior of the cocaine pharmacokinetics was studied using the researcher's own i.v. data and the intranasal and oral data reported by Javaid et al. (44) and Van Dyke et
al. (71). A strong positive correlation \((r=0.946)\) was demonstrated between dose and biological half-life, a strong negative correlation \((r=-0.973)\) between dose and plasma clearance and a weak negative correlation \((r=-0.890)\) for \(V_B\) with dose. This demonstration of dose-dependency of cocaine disposition, however, must be regarded as tentative because the data of Barnett et al. (78) were based on a small number of subjects and were pooled with data of other researchers who used different protocols and assay methodology.
Chapter II

RESEARCH OBJECTIVES

2.1 IDENTIFICATION AND ASSAY OF COCA ALKALOIDS

A number of gas chromatographic (GC) methods have become available for the determination of cocaine in coca leaves. Turner et al. (79) determined the cocaine content of leaf samples of *E. coca* collected from different locations in Peru. The assay procedure involved extraction of cocaine from powdered coca leaves with various solvents at reflux temperature and acid-base partitioning with chloroform followed by quantitation by GC. The most efficient extraction was obtained with a 15-min reflux with 95% ethanol. The use of 1.5% citric acid followed by sodium bicarbonate in the acid-base partitioning step resulted in quantitative recovery of cocaine. The cocaine content of leaf samples of *E. coca* was found to be 0.57 to 0.60%. Turner et al. (80) also analyzed leaf samples of *E. coca* for cis- and trans-cinnamoylcocaines content by a GC method. The percentage of cis-cinnamoylcocaine was 0.03-0.08%, and that of trans-cinnamoylcocaine 0.01-0.07%. Minimal concentrations of cocaine ranging from zero to 0.00141% have been found in 7 wild species of the genus *Erythroxylum* analyzed by GC (24).
Mass fragmentography has also been used to determine the cocaine content in various species of *Erythroxylum* (5, 8). The various methods for determining cocaine and its congeners in pharmaceutical preparations, street drug samples and biological fluids have been reviewed (81).

In the present study, m.p., IR and UV spectra will be examined to confirm the identity and purity of the cocaine hydrochloride required for the cocaine assays and the several chewing gum formulations. The assay procedure for cocaine in powdered leaves of *Erythroxylum*, *E. coca* and *E. novogranatense* var. *truxillense*, will consist of the extraction of cocaine with 95% ethanol at reflux temperature, clean-up by acid-base partitioning with methylene chloride, and quantification by GC. The GC assay employed in the present research for measuring cocaine in the various samples of cocaine and coca preparations (extracts and chewing gum formulations) makes use of flame-ionization detection. The n-propyl ester of benzoylcegonine (n-PEBE) will serve as the internal standard. While unnecessary for cocaine and n-PEBE¹, derivatization will be employed in the present GC procedure; bis(trimethylsilyl)-trifluoroacetamide (BSTFA) will be used as the derivatizing agent. This procedure will not interfere with the quantification of cocaine, but will allow the detection and measurement of any hydrolysis product.

¹ Cocaine and n-PEBE can not be derivatized by BSTFA but are sufficiently polar to be eluted off the GC column at operating oven temperature.
i.e., benzoylcagonine, ecgonine methyl ester or ecgonine which may be formed during the assay.

An ethanolic extract of two types of coca leaf will be prepared by solvent extraction followed by percolation. The cocaine content of the two extracts will be measured by GC. GC fingerprints of non-derivatized and BSTFA-derivatized samples of the two extracts will also be obtained, and the peaks of the GC tracing for each species compared. These fingerprints will serve as confirmatory aids in the standardization of the two coca extracts.

2.2 CHEWING GUM FORMULATIONS

Coca leaves have been used as a stimulant and medicinal in South America for thousands of years. The coca is taken by the natives of South America by mouth in the form of dried leaves and powders, or as a decoction for the treatment of a variety of ailments (15, 82). It is believed to be physiologically beneficial to the South American Indians in their adaptation to hunger, cold, and fatigue at high altitudes. It is frequently employed as a prophylactic or as a remedy among South American Indians for altitude sickness (82).

The symptoms of altitude or mountain sickness (18, 83) are similar to those of motion sickness, i.e., vertigo, nausea and vomiting. While the etiology of motion sickness
and the mechanism through which it occurs are poorly understood, the vestibular system within the inner ear is considered to be primarily involved in the occurrence of motion sickness (84). The neurons involved in vestibular reactions are cholinergic (85, 86).

A variety of motion sickness which is becoming increasingly important and receiving a great deal of attention is space motion sickness as experienced by space shuttle astronauts (87). Available remedies are seriously limited. The use of antimotion sickness drugs to prevent space motion sickness is accompanied by CNS and motor impairment produced as side effects of these medications.

Drugs which are effective in preventing motion sickness include certain antihistamines, belladonna, phenothiazines, amphetamines and barbiturates. Among these drugs scopolamine is the oldest and is one of the most effective (88). Scopolamine as well as other antimotion sickness drugs have some side effects including dry mouth and drowsiness which may cause loss of perceptual and operational proficiency. Ideally, remedies for the treatment of motion sickness should be effective and specific as well as being non-toxic and free from side effects. Mechanistically, an effective antimotion sickness drug should block acetylcholine in the CNS and also activate the central sympathetic area. A combination of drugs or single drug possessing these two activities is considered to be the near ideal preparation for preventing motion sickness (89).
The physiological activity resulting from coca chewing beneficial in altitude or mountain sickness is most likely due to the alkaloids in the coca plant. The coca alkaloids belong to the tropane series, the same group as atropine and scopolamine which come from plants of Solanaceae. It has been reported that cocaine, like atropine, inhibits the uptake of acetylcholine in rat brain cortex slices (90). Also, cocaine the most active of the coca alkaloids has a very powerful stimulant action on the CNS and potent effects on the sympathetic nervous system. Although according to present knowledge the ability of cocaine to enhance performance is uncertain, other drugs with sympathomimetic action have been shown to sustain a high level of proficiency (91). On this pharmacological basis coca leaf or possibly its active ingredient, cocaine, can be considered to have potential prophylactic or therapeutic value in the treatment of motion sickness. As a potentially useful therapeutic agent in motion sickness cocaine possesses certain other desirable attributes. It is rapidly absorbed from mucous membranes and also from the gastrointestinal tract after oral ingestion. Cocaine shows controlled sympathetic enhancement within minutes following application to mucous membranes (44, 71, 77).

The purpose of the present study is to design and formulate a novel oral dosage form of cocaine and coca alkaloids suitable for possible evaluation for the treatment of motion sickness. Chewing gum has been chosen as the most
suitable dosage form. As a coca-containing chewing gum, it would be most useful in simulation studies of coca leaf chewing. Furthermore, as a dosage form for use by astronauts in a zero-G environment, chewing gum enjoys certain special advantages over such conventional dosage forms as liquids, capsules and tablets. In space, chewing gum can be conveniently administered as a whole dosage unit, or as fractional units without the need for measuring devices or creating hazardous free-floating dust particles in space. Chewing gum can be easily retained in the oral cavity for continued drug release for pre-determined periods of time. Liquid dosage forms require sealed containers with special adaptors designed for drug delivery under conditions of zero-G to guard against accidental spillage. Commonly available sustained or prolonged release capsule or tablet formulations remain to be demonstrated as effective dosage forms in space. As a space dosage form, chewing gum represents the best approach for providing controlled rapid and prolonged absorption of active drugs across the oral mucosa and gastrointestinal tract. Administration of coca-chewing gum would most closely simulate the practice of coca leaf chewing.

The alkaloid content of coca leaves can vary greatly depending on the conditions under which the leaves are stored, such as temperature, humidity, molds and the duration of storage. The content of cocaine varies from leaf to leaf. To have a reliable and more uniform preparation for
the coca-chewing gum formulations, a standardized coca extract will be prepared from coca leaves. To facilitate the dispersion of the extract in the gum formulation, the coca extract will be adsorbed onto dextrin (producing coca-dextrin). For reference purposes, cocaine-dextrin will be formulated in a similar manner. The cocaine content of the coca- and cocaine-dextrins will be analyzed by the GC method previously described.

Various proportions of gum base, syrup, and water will be examined to obtain an optimum chewing gum formulation taking into consideration its kneadability and consistency. Novel procedures for the assay of cocaine in the cocaine- and coca-chewing gums will be developed. Special attention will be given to possible changes in cocaine due to its susceptibility to hydrolysis.

A bioavailability study of the cocaine- and coca-chewing gums in humans would provide an appropriate biopharmaceutical evaluation of the gum products. A number of legal and ethical considerations, however, make it difficult to undertake this type of study. Administration of cocaine to naive subjects is generally considered unethical. Volunteers with a history of cocaine use are acceptable but finding a sufficiently healthy drug-free sample population is also a difficult problem. In addition, lack of a suitable animal model for evaluating chewing gum as a dosage form makes it necessary to rely primarily on \textit{in vitro} release studies for
evaluation of the gum formulations produced in the present research.

The in vitro release profile of cocaine from chewing gum formulations will be studied using both water and artificial saliva at 37°C as test media. The release behavior of cocaine from the gum formulations will also be examined according to a pharmacokinetic model. The in vitro release studies are aimed at confirming the release of cocaine from the gum formulation and at characterizing quantitatively the release profile.
Chapter III
EXPERIMENTAL

3.1 COCAINE HYDROCHLORIDE

3.1.1 Materials and Equipment

Cocaine hydrochloride (Lot #84030, Code K 90780) was purchased from BDH Chemicals, Toronto, Ont., Canada, and samples removed for use were stored under vacuum over phosphorus pentoxide.

A Hewlett-Packard Model HP 5711 gas chromatograph (GC) equipped with a flame ionization detector was used for the analysis of cocaine. Coiled glass columns (2 mm x 2 m) pretreated with 5% dimethyldichlorosilane in toluene were packed with Chromosorb High Performance, 80/100 mesh, as the solid support coated with 3% OV-101. The columns were conditioned and maintained with periodic 25 µL injections of Silyl-8. The column materials and reagents were purchased from Chromatographic Specialties Ltd., Brockville, Ont., Canada. Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) which was used as the derivatizing agent was purchased from Regis Chemical Co., Morton Grove, Ill., U.S.A. The Silyl-8 and BSTFA were stored at 4°C. Methanol and 2-propanol were pesticide grade and other chemicals and solvents were reagent
grade. The n-propylester of benzoylecgonine (n-PEBE) which served as the internal standard in the gas chromatographic analysis of cocaine was synthesized¹ according to an established method for the synthesis of esters (92).

An Electrothermal Melting Point Apparatus, a Perkin Elmer Model 267 Grating Infrared Spectrophotometer and a Beckman UV Spectrophotometer ACTA III were used to confirm the identity and purity of cocaine hydrochloride samples.

A Modular Dri-Bath distributed by Thermolyne Corp., Dubuque, Iowa, U.S.A., was used to evaporate off solvent from extracts under a stream of helium gas, and to heat reaction mixtures with derivatizing reagents. When necessary, solutions and reaction mixtures were centrifuged in a Dynac Centrifuge distributed by Clay Adams, Parsippany, NJ., U.S.A.

3.1.2 Methods

Identification. The melting point of cocaine hydrochloride previously dried in vacuo over phosphorus pentoxide was determined and compared with its literature value.

For the IR spectral analysis, cocaine hydrochloride (1.6 g) and potassium bromide (300 mg), IR grade, were triturated and used to form a compressed disc. The IR spectra of the KBr disc was recorded between 4000 cm⁻¹ and 670 cm⁻¹. The position and relative intensities of the absorption max-

¹ Dr. T. G. Vitti performed the n-PEBE synthesis.
ima in the spectrum were compared to literature values.

The UV spectrum of cocaine hydrochloride dissolved in methanol (20 µg/mL) was recorded between 350 nm and 220 nm with a scan speed of 2 nm/s, chart expansion of 20 nm/in. and span of 1.0. The λmax of the spectrum obtained was compared with the literature λmax.

**Cocaine Assay.** Cocaine was assayed according to a modification of Moore's GC method (93). Conditions for GC operation during cocaine analysis included flow rates of nitrogen carrier gas at 40 mL/min, air at 240 mL/min and hydrogen at 30 mL/min. Both injector and detector ports were maintained at 300°C. The oven temperature was programmed from 135° to 270° at a rate of 16°/min, with the initial and final temperatures held for 2 min. The attenuator setting was 64 x 10 equivalent to 3.2 x 10⁻⁹A for full scale deflection, and the chart speed was set at 0.25 in. per min.

Stock standard solutions of cocaine hydrochloride containing 400 µg/mL of cocaine as free base and of n-PEBE, 200 µg/mL in methanol were prepared. Dilute hydrochloric acid (0.6 N) was prepared by diluting 5 mL of constant-boiling HCl (6.0 N) with 2-propanol to a volume of 50 mL. When not in use the standard solutions were stored at -15°C.

For the construction of the cocaine calibration curve 0, 50, 100 and 200 µL of the cocaine standard solution were added to four separate 12 mL screw-cap test tubes, and 25 µL
of 0.6 N HCl in 2-propanol added to each tube. To each tube 200 mL of the n-PEBE internal standard solution was added and the contents evaporated to dryness at 50°C under a stream of helium gas. After adding 50 µL of BSTFA, each tube was tightly capped and the contents mixed by means of a vortex mixer, and then heated at 70°C for 10 min. After cooling, the tubes were centrifuged at about 2,500 rpm for 5 min. About 1 µL of the contents of each tube was injected into the GC. A standard assay curve for cocaine was drawn by plotting the ratio of the cocaine peak to the n-PEBE peak versus concentration of cocaine. The slope and correlation coefficient of the line plot were determined from a least squares linear regression. The reproducibility of the slope and the correlation coefficient were checked routinely. A correlation coefficient greater than 0.995 was considered as acceptable.

3.2 COCA LEAF POWDER AND EXTRACTS

3.2.1 Materials and Equipment

Leaves of two species of the genus Erythroxylum, E. coca and E. novogranatense var. truxillense, were used as the plant source material for the extraction of coca alkaloids. The leaves were obtained from the Empresa Nacional De La Coca, Lima, Peru. A Thomas Wiley Mill, Model ED-5, distributed by Arthur H. Thomas Co., Philadel., PA., U.S.A. was used to grind the leaves.
An International Centrifuge, Model UV, distributed by International Equipment Co., Needham, MA., and a Dynac Centrifuge distributed by Clay Adams, Parsippany, NJ. were used for the separation of liquid-solid or liquid-liquid phases obtained in the assay and extraction procedures.

A glass column (44 mm x 335 mm) fitted with a coarse sintered-glass filter was used in the procedure for the extraction of alkaloids from the powdered coca leaves. Methylene chloride used for the extractions was reagent grade.

3.2.2 Methods

Preparations. Leaves of E. coca and E. novogranatense truxillense were carefully garbled and then ground in a cutter mill equipped with a 0.5 mm sieve.

Ethanolic extracts of the powdered leaves were prepared as follows: To 20.0 g of the powdered leaves in a 250 mL beaker were added 120.0 mL of boiling 95% ethanol and the mixture stirred. The beaker contents were covered with aluminium foil and placed in a sealed Styrofoam® container for 4 hours; once every hour the mixture was stirred for 1 min. The suspension was then poured into a glass column fitted with a sintered-glass filter at the bottom. The percolate was collected in a 500 mL Erlenmeyer flask. When the level of the ethanol layer in the column was about 1 cm from the top of the packed coca powder, an additional 40 mL of 95%
ethanol was added as a wash. The wash was repeated two more times with fresh ethanol. The several washes were added to the initial percolate. Sixty milliliters (60.0 mL) of 95% ethanol was further added to the column and allowed to stand in contact with the coca powder residue overnight. The solvent was then drained out of the column into the flask. The powder residue was washed twice with 80 mL portions of 95% ethanol, and the washes added to the bulk solution. The combined extract and washes were diluted to a volume of 500 mL with 95% ethanol. Both ethanolic extracts obtained from E. coca and E. novogranatense var. truxillense were stored at 4°C until analyzed by GC for cocaine content or characterized by GC fingerprinting, or used for the preparation of coca-dextrin. The residues of coca leaf fibre remaining after the ethanolic extraction were first dried for 2 days at reduced pressure in a vacuum dessicator over concentrated sulfuric acid and then analyzed for residual cocaine.

**Potency.** The cocaine content of each coca leaf powder preparation was determined by extracting the powdered leaf with 95% ethanol at reflux temperature, acid-base partitioning with methylene chloride followed by gas chromatography of the purified extract: About 100 mg of coca leaf powder was weighed accurately and placed into a 12 mL screw-cap test tube. The powder was extracted with 5 mL of 95% ethanol at 50°C for 10 min; the tube contents were mixed on a vortex mixer for 10 sec every 2 min during the extraction period,
and then centrifuged at a speed of 2,500 rpm for 10 min. The supernatant was transferred to a fresh tube. The residue was rinsed by mixing with 2.0 mL of 95% ethanol, and the resulting suspension centrifuged. The supernatant was added to the initial extract. The combined extracts were evaporated to dryness under a stream of helium gas at 50°C. Four milliliters (4.0 mL) of methylene chloride was added to dissolve the residue. After the addition of 3.0 mL of 1.5% (W/V) citric acid solution to the tube, the tube was shaken 10 times by hand followed by 5 min on the flat-bed shaker and then centrifuged at 2,500 rpm. Two milliliters (2.0 mL) of the aqueous phase was transferred to a fresh tube and 8.0 mL of methylene chloride added. After 250 mg of NaHCO₃ were slowly added to neutralize the acid, the tube was immediately hand-shaken 10 times, shaken 5 min on the flat-bed shaker and centrifuged. One milliliter (1.0 mL) of the organic phase was transferred to a fresh tube containing 100 μL of 0.6 N HCl in 2-propanol. The solvent mixture was evaporated to dryness under a stream of helium gas at 50°C and the residue subjected to gas chromatographic analysis for cocaine as previously described.

In the assay procedure for cocaine in the coca leaf powder preparations, cocaine calibration standards were prepared by adding 0.5, 1.0, 1.5 and 2.0 mL aliquots of cocaine standard solution (400 μg/mL) to each test tube containing 6.5, 6.0, 5.5 and 5.0 mL of 95% ethanol, respectively. The
samples were evaporated to dryness at 50°C under a stream of helium gas. Cocaine was extracted and analyzed as previously described for coca leaf powder.

For the analysis of the ethanolic extracts of E. *coca* and E. *novogranatense* var. *truxillense* for cocaine content, 2.5 mL aliquot samples were taken. To each sample was added 2.5 mL of 95% ethanol and the solvent evaporated under a stream of helium gas at 50°C. Cocaine was extracted from the residue with methylene chloride by acid-base partitioning and determined by the GC method previously described. To measure the residual cocaine content of the exhausted coca leaf fibres, 100 mg samples of each residue were taken and analyzed using the same procedure as used for the fresh coca leaf.

**GC Fingerprinting.** To obtain derivatized and non-derivatized GC finger prints of E. *coca* and E. *novogranatense* var. *truxillense* extracts, a 5.0 mL aliquot of each ethanolic extract was used. To each aliquot 95% ethanol was added to make a final volume of 100 mL. For the non-derivatized chromatogram, 4.0 mL of the diluted sample of each coca leaf extract were transferred to a test tube, and the sample taken to dryness at 50°C. A 1.0 mL rinse of 95% ethanol was added to the tube, and the tube contents centrifuged. About a 1 µL aliquot of the ethanolic sample was injected directly into the GC. For the derivatized chromatogram of the coca extract, 4.0 mL of the diluted sample of each coca extract
were added to a tube, and after evaporation of the solvent 50 μL of BSTFA were added. The tube was tightly capped, shaken on the vortex mixer, heated at 70°C for 10 min, and then centrifuged. About 1 μL of the BSTFA reaction mixture was injected into the GC. The initial and final oven temperatures were set at 100°C and 200°C, held for 0 and 2 min, respectively. The programmed rate of increase in oven temperature was 8°C/min. GC tracings of the non-derivatized and derivatized samples of each coca leaf species were compared.

3.3 CHEWING GUM FORMULATIONS

3.3.1 Materials and Equipment

Dextrin was purchased from ICN Nutritional Biochemicals, Cleveland, Ohio, U.S.A. A Büchi Rotavapor-RE (Brinkmann Instruments) was used for the preparation of cocaine- and coca-dextrin. Gum base was a gift from the L.A. Dreyfus Company of South Plainfield, NJ., and corn syrup was purchased from St. Lawrence Starch Company, Ltd., Mississauga, Ont., Canada. Gum base, corn syrup, water and glycerin (Fisher) were used as the ingredients for the cocaine- and coca-chewing gum formulations.

Chewing gum was prepared in a 125 mL stainless steel beaker installed in a boiling-water bath. For molding the chewing gum into sticks, a stainless steel roll plate (155 mm x 255 mm x 10 mm) and a stainless steel rod (12 mm x 480 mm) were used (Fig.3). A 50 mL test tube (25 mm x 200 mm)
Figure 3: Stainless-steel plate (a) and rolling pin (b) used to roll chewing gum bolus into sheets of uniform thickness.
tightly fitted with a rubber stopper at the round bottom was used as vessel for the *in vitro* release study of cocaine from cocaine- and coca-chewing gums. A glass rod (6 mm x 250 mm) with one blunt end was used to knead the gum to simulate *in vivo* mastication. Artificial saliva and water were used as test media for the release studies. Artificial saliva based on a modification of that given by Shannon et al. (94) was made up according to the formula shown in Table 1. The potassium chloride, sodium chloride, potassium phosphate (both monobasic and dibasic), as well as the magnesium chloride, calcium chloride and sorbitol were reagent grade.

Cocaine release studies were done at 37°C using a water bath in which the temperature was kept constant by means of a temperature-controlled circulating/heating pump distributed by Precision Scientific Group, Chicago, Ill., U.S.A.
TABLE 1

Composition of artificial saliva\textsuperscript{1} used for \textit{in vitro} release studies of cocaine from cocaine- and coca-chewing gums.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g or mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>1.249</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.731</td>
</tr>
<tr>
<td>MgCl(_2)_6H(_2)O</td>
<td>0.188</td>
</tr>
<tr>
<td>CaCl(_2) Anhydrous</td>
<td>0.333</td>
</tr>
<tr>
<td>K(_2)HPO(_4)</td>
<td>1.607</td>
</tr>
<tr>
<td>KH(_2)PO(_4)</td>
<td>0.652</td>
</tr>
<tr>
<td>70% sorbitol</td>
<td>85.5</td>
</tr>
<tr>
<td>Na carboxymethyl cellulose</td>
<td>20.0</td>
</tr>
<tr>
<td>Water, q.s. ad</td>
<td>2000.0</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Based on saliva substitute of Shannon et al. (94).
3.3.2 Methods

3.3.2.1 Cocaine- and Coca-Dextrin

Preparation. Cocaine hydrochloride equivalent to 1.300 g free base was weighed out and placed into a 1-L Büchi flask. Fifty milliliters (50.0 mL) of 95% ethanol were added to the flask to dissolve the cocaine hydrochloride. Dextrin (55.00 g) was added to the flask followed by 50.0 mL of ethanol as a rinse. With the flask attached to the vacuum evaporator, the ethanol was distilled off under reduced pressure at 50°C causing the cocaine hydrochloride to be adsorbed onto the dextrin. The flask contents were then allowed to stand in open-air for one hour for evaporation of most of the remaining ethanol. The resulting solid mass of cocaine-dextrin was scraped out of the flask, and put into a jar for drying at reduced pressure over concentrated sulfuric acid. The cocaine-dextrin material was triturated in a mortar and passed through a 30 mesh sieve, and dried to constant weight at reduced pressure over concentrated sulfuric acid in a desiccator.

For the preparation of coca-dextrin, an ethanolic extract of powdered leaves of _E. novogranatense_ var. _truxillense_ equivalent to approximately 220 mg of cocaine was adsorbed onto 22.0 g of dextrin in a similar manner as for cocaine dextrin.
Cocaine Potency. About 100 mg of cocaine- or coca-dext- 
rin was weighed out accurately and the cocaine content mea-
ured in a similar manner as used for the analysis of cocaine 
in coca leaf powder.

3.3.2.2 Cocaine- and Coca-Chewing Gums

Preparation. In initial experiments chewing gums con-
taining cocaine-free dextrin were first formulated with the 
aim of determining the gum composition offering optimum 
kneadability and consistency. A series of different formula-
tions with various proportions of the ingredients: gum base, 
corn syrup, dextrin, water and 2% methyl cellulose solution 
as shown in Table 2 were tested.

Chewing gum was formulated as follows: Gum base was 
softened to near melting in a stainless steel beaker placed 
in a boiling water bath, and then mixed with corn syrup, wa-
ter or 2% methyl cellulose solution. Dextrin was added to 
the mixture and kneaded at 80-90°C until all ingredients 
were uniformly distributed. While allowed to cool gradually, 
the kneaded gum was rolled into a sheet on a stainless steel 
roll-plate (Fig. 3) and cut into rectangular pieces (20 mm x 
50 mm x 2 mm).

On the basis of the results obtained with the cocaine-
free chewing gum formulation No. 14 given in Table 2, co-
caine- and coca-chewing gum formulations were prepared with 
a slight modification of the relative proportions of ingre-
TABLE 2

Percent (%) composition of formulations screened to select optimum cocaine-free dextrin-containing chewing gum.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Gum base</th>
<th>Water</th>
<th>Corn syrup</th>
<th>Dextrin</th>
<th>MC° (2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>60</td>
<td></td>
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<td>26</td>
<td>13</td>
<td>14</td>
<td>47</td>
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</table>

°MC = methyl cellulose
dients including a small amount of glycerin (Table 3). Gum base (7.8 g) was melted in a stainless steel beaker placed in a boiling water bath. Corn syrup (3.9 g), glycerin (0.3 g) and water (3.9 mL) were added to the gum base. Cocaine- or coca-dextrin (14.0 g) was then added slowly, and the mixture kept at 80-90°C was thoroughly kneaded. The heated gum was rolled into a sheet while cooling on a stainless steel roll-plate designed to form 2 mm thick gum sticks. While still warm, the semi-solid gum was cut into rectangular pieces (20 mm x 50 mm x 2 mm). After cooling the gum sticks were wrapped in aluminum foil, sealed in a jar and kept at 4°C until ready for cocaine analysis or in vitro cocaine release evaluation.

Cocaine Potency. For the analysis of the cocaine- and coca-chewing gum preparations for cocaine content approximately 100 mg of gum stick were weighed out accurately. The sample was immersed under 5.0 mL of 95% ethanol in a mortar. The mortar and contents together with pestle were covered with parafilm and kept at -15°C in the deep-freeze for 1 h. While still cold, the gum sample was triturated in the mortar for 10 min, and then returned to the deep-freeze for an additional 1 h. This cooling-trituration cycle was repeated two more times or until a fine suspension was obtained. The suspension was transferred to a test tube, and the mortar and pestle washed with successive 3 mL and 2 mL portions of cold 95% ethanol. These washes were added to
**TABLE 3**

Percent (%) composition of final formulation used for the preparation of cocaine- and coca-chewing gums.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gum base</td>
<td>26</td>
</tr>
<tr>
<td>Corn syrup</td>
<td>13</td>
</tr>
<tr>
<td>Cocaine- or Coca-dextrin</td>
<td>47</td>
</tr>
<tr>
<td>Water</td>
<td>13</td>
</tr>
<tr>
<td>Glycerin</td>
<td>1</td>
</tr>
</tbody>
</table>
the tube. The tube contents were heated at 60°C for 10 min with intermittent shaking on a vortex mixer for 20 seconds every 2 min. The tube was centrifuged at 2,500 rpm for 10 min, and the supernatant transferred to a fresh test tube. Three milliliters (3.0 mL) of 95 % ethanol were added as a wash to the gum residue. The tube was shaken well on the vortex and again centrifuged for 10 min. The supernatant was added to the test tube containing the initial supernatant. The combined supernatants were evaporated to dryness at 50°C with a stream of helium gas. Cocaine was extracted from the residue by acid-base partitioning with methylene chloride and analyzed by the GC method as previously described.

In-vitro Release Profile. The in vitro release profile of cocaine was determined for cocaine- and coca-chewing gums in both water and artificial saliva. The artificial saliva, a modification of the saliva substitute developed by Shannon et al.(94), was prepared as follows: To sufficient water heated to approximately 50°C was added all the ingredients consisting of the potassium phosphates, the chlorides of potassium, sodium, magnesium and calcium and the 70 % sorbitol solution. Almost all of solids were dissolved with vigorous mixing. The resulting opalescent mixture was clarified by centrifugation for 15 min at 2,500 rpm. The clear supernatant was collected and kept at 4°C. The pH of the artificial saliva was 7.04.
Before conducting the in vitro release studies the recovery of cocaine from artificial saliva was examined. Measured aliquots (0.5, 1.0, and 2.0 mL) of cocaine standard solution (400 μg/mL) were added to separate test tubes and evaporated to dryness at 50°C under a stream of helium gas. Two milliliters (2.0 mL) of artificial saliva were added to each tube and vortexed well. Cocaine was extracted from the artificial saliva with 8 mL of methylene chloride and subjected to GC analysis as previously described. The amounts of cocaine (μg) found in the injected samples were compared with the theoretical amounts (μg) of cocaine expected.

For the in vitro release studies the specially designed test tube was filled with 20.0 mL of water or artificial saliva, and placed in a water bath at 37°C. One stick of cocaine- or coca-chewing gum was placed in the test tube and allowed to remain immersed and undisturbed for 30 seconds. For the next 5 min it was then kneaded with a glass rod to form a quid. Subsequently, the gum was kneaded at a rate of 20 times per min for 5 min. After the first 10 min operation the gum was taken out and rinsed with the test medium. The gum was kneaded for five additional 10 min-release periods, each time in fresh medium (20 mL). An aliquot of the test medium used for each 10 min period was transferred to separate tubes and saved for analysis of cocaine content. The spent gum was placed in a plastic vial and kept at -20°C until analyzed for cocaine. For cocaine analysis, 1.0 mL of
each test medium used for the release study was placed in a tube. Eight milliliters (8.0 mL) of methylene chloride were added, and the tube was shaken on the flat-bed shaker for 5 min for the extraction of cocaine, followed by centrifugation for 5 min. Cocaine content was determined by the GC method previously described. The release profiles of cocaine from cocaine- and coca-chewing gums in water and artificial saliva were compared. The spent chewing gum was analyzed for cocaine in a similar manner as previously described for the analysis of fresh, intact gum.

Data obtained on the in vitro release of cocaine from cocaine- and coca-chewing gums in water and artificial saliva were analyzed according to the pharmacokinetic model:

\[
\begin{align*}
\text{Gum (1)} & \quad \text{Medium (2)} \\
X_1 & \quad X_2 \\
\end{align*}
\]

where:

\[X_1 = \text{the amount of cocaine present in the chewing gum (1) at } t = t.\]

\[X_0 = \text{the original amount of cocaine present in the gum at } t = 0.\]

\[X_2 = \text{the amount of cocaine released into or present in the medium (2) at } t = t.\]

\[K = \text{the first-order rate constant for the release of cocaine into the medium.}\]
It was assumed that the release of cocaine is a first-order process in which Fick's Law of Diffusion applies:

\[-dX_1/dt = KX_1\]

Accordingly, the amount of cocaine, \( X_1 \), left in the gum at any time, \( t \), is given by the equation:

\[ X_1 = X_1^0 \exp(-Kt) \tag{1} \]

while the amount of cocaine present in the medium at any time, \( t \), is given by the equation:

\[ X_2 = X_1^0 [1 - \exp(-Kt)] \tag{2} \]

Also, when \( t = \infty \),

\[ X_2^* = X_1^0 \tag{3} \]

Experimentally, equation (2) is more useful than equation (1) for the evaluation of pharmacokinetic parameters. Combining equations (2) and (3) yields

\[ (X_2^* - X_2) = X_1^0 \exp(-Kt) \tag{4} \]

In log form, equation (4) becomes:

\[ \log(X_2^* - X_2) = \log X_1^0 - Kt/2.303 \tag{5} \]

According to equation (5), the release rate constant, \( K \), can be calculated from the slope of the least squares linear regression obtained by plotting the logarithm of the amount of cocaine in the chewing gum to be released, \( \log(X_2^* - X_2) \),
against time, $t$. The value used for $X^T$ in the calculations corresponds to the asymptotic value of the amount of cocaine ultimately released into the water or artificial saliva. The present approach for calculating $K$ corresponds essentially to the "sigma-minus" method for calculating pharmacokinetic parameters.
Chapter IV
RESULTS

4.1 COCAINE HYDROCHLORIDE

Melting point and IR and UV spectrophotometric spectra confirmed the identity and purity of the cocaine hydrochloride used in the present research. The melting point of 196°C compared favorably with the literature value (27, 33, 34). The IR spectrum (Fig. 4) showed peaks identical to those published for cocaine (95). The UV spectrum of cocaine hydrochloride (Fig. 5) showed maximum absorption peaks at 230, 274, and 281 nm, characteristic of cocaine (81).

A modification of the GC method of Moore (93) was successfully used for determining the cocaine potency of the several cocaine-containing preparations in the present research. It included the use of n-PEBE as internal standard. As can be seen from the sample chromatogram in Fig. 6, n-PEBE is a suitable internal standard for cocaine. n-PEBE elutes after cocaine with a retention time of 8.6 min, as compared with 7.9 min for cocaine.

A standard concentration curve for increasing amounts of cocaine injected into the GC was constructed over the range from 0.4 to 1.6 µg by plotting the ratios of the co-
Figure 4: IR spectrum of sample of cocaine hydrochloride [British Drug House (BDH), Lot #84030, Code K 90780] in KBr pellet.
Figure 5: UV absorption spectrum of sample of cocaine hydrochloride (BDH, Lot #84030, Code K 90780) in methanol, 20 µg/mL.
Figure 6: Typical GC-assay tracings of BSTFA solvent blank (A) and cocaine standard with n-propyl ester of benzoylcegonine (n-PEBE) as internal standard (B). Key: (a) cocaine, (b) n-PEBE. [BSTFA = Bis(tri-methylsilyl)-trifluoroacetamide]
caine peaks to the n-PEBE peak, as shown in Fig. 7. The data yielded a straight line plot with a near zero-intercept. Linear regression analysis of the data points indicated a correlation coefficient of 1.000. The mean coefficient of variation (CV) for the samples at the three different concentrations of cocaine was 2.76%. Under the experimental conditions described, the minimal detectable quantity of cocaine was 0.017 µg.

4.2 COCA LEAF POWDER AND EXTRACTS

Powdered leaf samples of the two species of the genus *Erythroxylum*, *E. coca* and *E. novogranatense* var. *truxillense*, were analyzed for cocaine content. Typical GC chromatograms obtained for the assay of the powdered leaves are presented in Fig. 8. Besides the cocaine and internal standard (n-PEBE) peaks, several other minor peaks appear in the chromatograms. These extraneous peaks, however, are well separated and do not interfere with the measurement of cocaine. For the most part the minor peaks have similar retention times in the two chromatograms.

Recovery of cocaine from spiked aqueous solutions used as concentration standards in the coca leaf powder extraction-assay procedure are shown in Fig. 9. Quantitative recovery of cocaine was achieved over the concentration range from 0.3 to 1.5 µg/mL using 1.5% citric acid solution and an excess of NaHCO₃ in the acid-base partitioning step. The
Figure 7: GC-calibration curve for cocaine: cocaine peak/n-PEBE peak height ratio vs. amount of cocaine (µg) injected into GC.
Figure 8: Typical GC-assay tracings of leaf extracts of *E. coca* (A) and of *E. novogranatense* var. *truxillense* (B). Key: (a) cocaine, (b) n-PEBE (internal standard).
Figure 9: Recovery of cocaine from spiked aqueous standards subjected to extraction/assay procedure used for coca leaf powder. Slope of least squares regression line (recovery) equals 0.9835, r=0.9999.
extraction efficiency calculated from the least squares linear regression line was 98.35% with a correlation coefficient of 1.000.

The cocaine content of the powdered leaves of the two species of Erythroxylum is presented in Table 4. The mean value of three determinations was 0.435% (CV=0.96%) cocaine for E. coca and 0.481% (CV=3.87%) for E. novogranatense var. truxillense on a dry weight basis.

Cocaine concentration of the 500 mL-ethanolic extracts of E. coca and of E. novogranatense var. truxillense prepared by percolation of coca leaf powder and that of the exhausted fibre residues as analyzed by GC are presented in Table 5. The cocaine concentration obtained as a mean of 3 determinations was 0.502% (CV=0.24%) and 0.580% (CV=1.67%) for the extracts of E. coca and E. novogranatense var. truxillense, respectively, and 0.05 and 0.07% for their respective exhausted fibre residues.

The GC fingerprints of derivatized and non-derivatized samples of ethanolic extracts of E. coca are presented in Fig. 10 and 11, respectively. The corresponding GC fingerprints for E. novogranatense var. truxillense are presented in Fig. 12 and 13. The heights of some characteristic peaks in the GC tracing of derivatized and non-derivatized samples of each species are compared in Table 6 and 7. Peak heights less than 5 mm were not recorded.
**TABLE 4**

Cocaine content of powdered coca leaf of two species of the genus *Erythroxylum*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cocaine Content (%)&lt;sup&gt;1&lt;/sup&gt;</th>
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<tbody>
<tr>
<td><em>E. coca</em></td>
<td>0.435 ± 0.004</td>
</tr>
<tr>
<td><em>E. novogranatense var. truxillense</em></td>
<td>0.481 ± 0.019</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mean ± std. dev. of triplicate determinations on a dry weight basis.
TABLE 5

Cocaine content of ethanolic extracts and fibre residue from powdered leaves of *E. coca* and *E. novogranatense* var. *truxilliense*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cocaine Content (%)(^1)</th>
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<tbody>
<tr>
<td></td>
<td>Ethanolic Extract(^2)</td>
<td>Fibre Residue</td>
<td></td>
</tr>
<tr>
<td><em>E. coca</em></td>
<td>0.502 ± 0.001</td>
<td>0.053 ± 0.000</td>
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<tr>
<td><em>E. novogranatense</em> var. <em>truxilliense</em></td>
<td>0.580 ± 0.010</td>
<td>0.071 ± 0.010</td>
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</table>

\(^1\) Mean ± std. dev. of triplicate determinations.

\(^2\) Final volume = 500 mL.
Figure 10: Typical GC-fingerprint of BSTFA-derivatized residue from sample of ethanolic extract of E. coca leaves. [BSTFA = Bis(trimethylsilyl)-trifluoroacetamide]
Figure 11: Typical GC-fingerprint of non-derivatized residue from sample of ethanolic extract of *E. coca* leaves.
Figure 12: Typical GC-fingerprint of BSTFA-derivatized residue from sample of ethanolic extract of *E. novogranatense* var. *truxillense* leaves. [BSTFA = Bis(trimethylsilyl)-trifluoroacetamide]
Figure 13: Typical GC-fingerprint of non-derivatized residue from sample of *E. novogranatense var. truxillense* leaves.
TABLE 6
Comparison of characteristic peaks found in GC-fingerprints of BSTFA-derivatized and non-derivatized residues from samples\(^1\) of ethanolic extracts of E. coca leaves.

<table>
<thead>
<tr>
<th>Retention Time (min)</th>
<th>Peak Height (mm)</th>
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<tbody>
<tr>
<td>1.08</td>
<td>17.0</td>
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<tr>
<td>2.68</td>
<td>68.6</td>
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<tr>
<td>4.48</td>
<td>(\uparrow^3)</td>
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<tr>
<td>5.84</td>
<td>35.6</td>
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<td>8.64</td>
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<tr>
<td>9.12</td>
<td>36.5</td>
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<tr>
<td>9.52</td>
<td>76.4</td>
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<tr>
<td>9.80</td>
<td>160.5</td>
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<td>10.00</td>
<td>70.0</td>
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<td>71.0</td>
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<td>52.8</td>
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<tr>
<td>11.84</td>
<td>33.0</td>
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<tr>
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<td>18.16</td>
<td>46.7</td>
</tr>
<tr>
<td>18.76</td>
<td>29.0</td>
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</table>

\(^1\)Equivalent to 160 \(\mu\)g of dry coca leaf powder.

\(^2\)Peak height less than 5 mm.

\(^3\)Peak height off scale.
TABLE 7

Comparison of characteristic peaks found in GC-fingerprints of BSTFA-derivatized and non-derivatized residues from samples of ethanolic extracts of *E. novogranatense* var. *truxilense* leaves.

<table>
<thead>
<tr>
<th>Retention Time (min)</th>
<th>Peak Height (mm)</th>
<th>Derivatized Extract</th>
<th>Non-derivatized Extract</th>
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<tr>
<td>2.68</td>
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<td>13.4</td>
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<td>43.4</td>
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<td>13.52</td>
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<td>17.68</td>
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<td>18.16</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18.76</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

1Equivalent to 160 µg of dry coca leaf powder.
2Peak height less than 5mm.
3Peak height off scale.
4.3 CHEWING GUM FORMULATIONS

Cocaine-dextrin and coca-dextrin were successfully formulated by adsorption of cocaine hydrochloride or the residue of an ethanolic extract of powdered leaves of E. novogranatense var. truxillense onto dextrin. As shown in Table 8, the cocaine content of the cocaine- and coca-dextrin preparations measured was 2.25% and 0.98%, respectively.

The chewing gum formulations containing cocaine-free dextrin were first prepared taking into account the kneadability and consistency of each formulation (Table 9). The kneadability is referred to as the ease with which the bolus gum can be manually deformed by the application of physical pressure without deaggregation of the bolus, and the consistency as the relative balance between the amorphous and granular texture of the gum bolus. The preparations of cocaine- and coca-chewing gums were based on the composition of the formulation shown in Table 3. Glycerin was added to the formulation for its water-retaining properties. Although both gums were prepared using the same formulation, the coca-chewing gum showed a softer consistency than cocaine-chewing gum. The cocaine content of the cocaine- and coca-chewing gums was 1.08% and 0.45%, respectively (Table 10).

In preparation for the in vitro release studies of cocaine from cocaine- and coca-chewing gums in water and artificial saliva as test media, the recovery of cocaine by ex-
<table>
<thead>
<tr>
<th></th>
<th>Cocaine Content (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± Std. Dev.</td>
<td>Coeff. Var. (%)</td>
<td></td>
</tr>
<tr>
<td>Cocaine-dextrin</td>
<td>2.253 ± 0.071</td>
<td>3.14</td>
<td></td>
</tr>
<tr>
<td>Coca-dextrin</td>
<td>0.975 ± 0.010</td>
<td>1.07</td>
<td></td>
</tr>
</tbody>
</table>

1Based on four replicate determinations.
TABLE 9

Kneadibility and consistency of different chewing gum formulations containing *cocaine-free* dextrin.

<table>
<thead>
<tr>
<th>Formulation No.</th>
<th>Kneadibility¹</th>
<th>consistency²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>2</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>3</td>
<td>*</td>
<td>***</td>
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<td>13</td>
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<td>*<strong>/</strong></td>
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<tr>
<td>14</td>
<td>***</td>
<td>**</td>
</tr>
</tbody>
</table>

¹Kneadibility: Ease with which the bolus gum can be manually deformed by the application of physical pressure without deaggregation of the bolus. Key: * very difficult, ** difficult, *** easy, **** very easy.

²Consistency: Relative balance between amorphous and granular texture of the gum bolus. Key: * viscous/soft/sticky texture, ** amorphous/semisolid, *** highly granular/hard solid.
TABLE 10
Cocaine content of cocaine- and coca-chewing gum formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Cocaine Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Theoretical(^1)</td>
</tr>
<tr>
<td>Cocaine-chewing gum</td>
<td>1.059</td>
</tr>
<tr>
<td>Coca-chewing gum</td>
<td>0.458</td>
</tr>
</tbody>
</table>

\(^1\)(Percent cocaine in cocaine or coca-dextrin) \times (Percent dextrin as cocaine- or coca-dextrin used in chewing gum formulation) + 100.

\(^2\)Based on duplicate determinations.
traction from artificial saliva samples spiked with increasing amounts of cocaine was first determined. In Fig. 14, the amount of cocaine recovered by extraction is compared with the theoretical amount expected. The slope of the least squares regression line, corresponding to a measure of recovery, was 0.970 with a correlation coefficient of 0.996. The mean coefficient of variation of the amount of cocaine found in all samples was 4.12%.

GC-assay tracings of extracted artificial saliva samples obtained from the in vitro release studies of cocaine- and coca-chewing gums are presented in Fig. 15. As can be seen in Fig. 15, several other peaks as well as cocaine and n-PEBE (added as internal standard) were observed in the chromatograms. These peaks were minor in size and did not interfere with the GC analysis of cocaine.

Results from two in vitro release studies of cocaine from cocaine-chewing gums both in water and artificial saliva are presented in Fig. 16. The results for the coca-chewing gums are presented in Fig. 17. The release profile of cocaine was reproducible for both cocaine- and coca-chewing gums when studied in the same medium, water or artificial saliva. Periodic and cumulative cocaine release data for the cocaine- and coca-chewing gums are presented in Table 11 and 12, respectively. Cumulative percent cocaine released from cocaine-chewing gum during the 80-min release period was 34.6% and 89.2% in water and artificial saliva, respective-
Figure 14: Recovery of cocaine from spiked artificial saliva samples subjected to extraction/assay procedure used for in vitro release studies. Slope of least squares regression line (recovery) equals 0.9696, r=0.9913.
Figure 15: Typical GC-assay tracings of artificial saliva samples obtained from in vitro release studies of cocaine-chewing gum (A) and coca-chewing gum (B). Key: (a) cocaine, (b) n-PEBE (internal standard).
Figure 16: Comparison of in vitro release profiles of cocaine from cocaine-chewing gum in distilled water (DW) and artificial saliva (AS). Key: △,○ data from study No. 1; ▲,● data from study No. 2.
Figure 17: Comparison of in vitro release profiles of cocaine from coca-chewing gum in distilled water (DW) and artificial saliva (AS). Key: △,○ data from study No. 1; ▲,● data from study No. 2.
**TABLE 11**

In vitro release study of cocaine from cocaine-chewing gum in distilled water and in artificial saliva at 37°C.

<table>
<thead>
<tr>
<th>Release Period (min)</th>
<th>Distilled Water % cocaine released(^1)</th>
<th>Cumulative % cocaine released</th>
<th>Artificial Saliva % cocaine released(^1)</th>
<th>Cumulative % cocaine released</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>18.20 ± 1.18</td>
<td>18.20</td>
<td>48.45 ± 1.11</td>
<td>48.45</td>
</tr>
<tr>
<td>10-20</td>
<td>9.48 ± 0.46</td>
<td>27.67</td>
<td>29.15 ± 0.70</td>
<td>77.59</td>
</tr>
<tr>
<td>20-30</td>
<td>3.55 ± 0.64</td>
<td>31.22</td>
<td>5.77 ± 0.94</td>
<td>83.36</td>
</tr>
<tr>
<td>30-40</td>
<td>1.30 ± 0.39</td>
<td>32.52</td>
<td>2.35 ± 0.21</td>
<td>85.72</td>
</tr>
<tr>
<td>40-50</td>
<td>0.64 ± 0.05</td>
<td>33.16</td>
<td>1.44 ± 0.29</td>
<td>87.16</td>
</tr>
<tr>
<td>50-60</td>
<td>0.57 ± 0.14</td>
<td>33.73</td>
<td>0.85 ± 0.28</td>
<td>88.01</td>
</tr>
<tr>
<td>60-80</td>
<td>(0.87 ± 0.03(^2))</td>
<td>34.60</td>
<td>1.17 ± 0.22</td>
<td>89.19</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± std. dev. based on duplicate samples from each of two separate experiments.

\(^2\) Mean ± std. dev. based on duplicate samples from one experiment.
**TABLE 12**

*In vitro* release study of cocaine from coca-chewing gum in distilled water and in artificial saliva at 37°C.

<table>
<thead>
<tr>
<th>Release period (min)</th>
<th>Distilled Water</th>
<th>Artificial Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% cocaine Released&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Cumulative % cocaine Released</td>
</tr>
<tr>
<td>0-10</td>
<td>71.61 ± 1.51</td>
<td>71.61</td>
</tr>
<tr>
<td>10-20</td>
<td>1.43 ± 0.71</td>
<td>73.04</td>
</tr>
<tr>
<td>20-30</td>
<td>0.38 ± 0.17</td>
<td>73.42</td>
</tr>
<tr>
<td>30-40</td>
<td>0.32 ± 0.13</td>
<td>73.74</td>
</tr>
<tr>
<td>40-50</td>
<td>0.31 ± 0.10</td>
<td>74.04</td>
</tr>
<tr>
<td>50-60</td>
<td>0.28 ± 0.10</td>
<td>74.32</td>
</tr>
<tr>
<td>60-80</td>
<td>0.30 ± 0.11</td>
<td>74.62</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mean ± std. dev. based on duplicate samples from each of two separate experiments.
ly, whereas from coca-chewing gum it was 74.6% and 92.1%, respectively.

The spent gum from the in vitro release studies of cocaine- and coca-chewing gums was analyzed for cocaine. The remaining cocaine content is presented in Table 13. Total amount of cocaine recovered during the 80-min release study and the remaining amount found in the spent cocaine- and coca-chewing gums are shown in Table 14. The mean total recovery of cocaine in the release studies of cocaine-chewing gum performed in water and in artificial saliva was 52.2% and 97.7%, respectively. The values for coca-chewing gum in water and artificial saliva were 75.8% and 92.0%, respectively.

The first-order rate constant for the release of cocaine from cocaine- and coca-chewing gums was determined by the so-called "sigma-minus" method. The required experimental data corresponding to the amounts of cocaine remaining to be released over time from the respective gum formulations into water or artificial saliva are presented in Table 15 and 16 for cocaine-chewing gum and in Table 17 and 18 for coca-chewing gum. The release rate constants calculated from the linear regression lines of these data are summarized in Table 19. The mean release rate constants of cocaine for cocaine-chewing gum in water and in artificial saliva were 0.071 min⁻¹ and 0.073 min⁻¹, respectively. The mean release rate constant of cocaine from coca-chewing gum...
TABLE 13

Comparison of cocaine content of original and spent cocaine- and coca-chewing gum samples used in the in vitro release studies.

<table>
<thead>
<tr>
<th>Gum Form.</th>
<th>Release Study</th>
<th>Original Gum Stick</th>
<th>Spent Gum Material</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wt. (g)</td>
<td>(mg) (%)</td>
</tr>
<tr>
<td>Cocaine-Chewing</td>
<td>DW #1</td>
<td>3.15</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>DW #2</td>
<td>3.26</td>
<td>35.1</td>
</tr>
<tr>
<td></td>
<td>AS #1</td>
<td>3.42</td>
<td>36.8</td>
</tr>
<tr>
<td></td>
<td>AS #2</td>
<td>3.30</td>
<td>35.5</td>
</tr>
<tr>
<td>Coca-Chewing</td>
<td>DW #1</td>
<td>3.00</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>DW #2</td>
<td>3.23</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>AS #1</td>
<td>3.38</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td>AS #2</td>
<td>3.30</td>
<td>14.9</td>
</tr>
</tbody>
</table>

1DW = distilled water, AS = artificial saliva.
2Mean of duplicate assays.
TABLE 14

Overall recovery of cocaine from cocaine- and coca-chewing gum formulations used in the 80-min *in vitro* release studies.

<table>
<thead>
<tr>
<th>Gum Form.</th>
<th>Release Studies¹</th>
<th>Test Medium (A)</th>
<th>Spent Gum (B)</th>
<th>Total (A + B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine-Chewing Gum</td>
<td>DW #1</td>
<td>11.5 (34.05%)²</td>
<td>6.5 (19.04%)³</td>
<td>18.0 (53.09%)</td>
</tr>
<tr>
<td></td>
<td>DW #2</td>
<td>12.1 (34.30%)</td>
<td>6.0 (16.94%)</td>
<td>18.0 (51.23%)</td>
</tr>
<tr>
<td></td>
<td>AS #1</td>
<td>32.2 (87.52%)</td>
<td>3.1 (8.44%)</td>
<td>35.3 (95.96%)</td>
</tr>
<tr>
<td></td>
<td>AS #2</td>
<td>32.2 (90.85%)</td>
<td>3.1 (8.62%)</td>
<td>35.3 (99.47%)</td>
</tr>
<tr>
<td>Coca-Chewing Gum</td>
<td>DW #1</td>
<td>10.4 (76.87%)</td>
<td>0.2 (1.27%)</td>
<td>10.6 (78.14%)</td>
</tr>
<tr>
<td></td>
<td>DW #2</td>
<td>10.6 (72.37%)</td>
<td>0.2 (1.01%)</td>
<td>10.7 (73.37%)</td>
</tr>
<tr>
<td></td>
<td>AS #1</td>
<td>13.9 (91.20%)</td>
<td>-</td>
<td>13.9 (91.20%)</td>
</tr>
<tr>
<td></td>
<td>AS #2</td>
<td>13.8 (92.89%)</td>
<td>-</td>
<td>13.8 (92.89%)</td>
</tr>
</tbody>
</table>

¹ DW = distilled water, AS = artificial saliva.
² Cumulative % cocaine released into medium over 80-min release period.
³ (Amount of cocaine found in spent gum) + (amount of cocaine in original gum) x 100
TABLE 15

Estimation of the first-order release rate constant, K, from a plot of the amount of cocaine in cocaine-chewing gum remaining to be released into water as test medium versus time.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Release Period (min)</th>
<th>$\Delta X_2$ (mg)</th>
<th>$\sum X_2$ (mg)</th>
<th>$(X_2^0 - X_2)$ (mg)</th>
<th>$K$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-10</td>
<td>6.47</td>
<td>6.47</td>
<td></td>
<td>5.06</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>3.28</td>
<td>9.75</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>1.01</td>
<td>10.76</td>
<td>0.77</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>0.33</td>
<td>11.09</td>
<td>0.44</td>
<td>(r=-0.994)</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>0.20</td>
<td>11.29</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>0.24</td>
<td>11.53</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0-10</td>
<td>6.06</td>
<td>6.06</td>
<td>6.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>3.31</td>
<td>9.37</td>
<td>2.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>1.44</td>
<td>10.81</td>
<td>1.27</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>0.57</td>
<td>11.39</td>
<td>0.70</td>
<td>(r=-0.992)</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>0.24</td>
<td>11.62</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>0.16</td>
<td>11.78</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80</td>
<td>0.31</td>
<td>12.09</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

$^1 X_2^0 \approx 11.53 \text{ mg cocaine for experiment No. 1, }$
$12.09 \text{ mg for Experiment No. 2.}$
TABLE 16

Estimation of the first-order release rate constant, K, from a plot of the amount of cocaine in cocaine-chewing gum remaining to be released into artificial saliva as test medium versus time.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Release Period (min)</th>
<th>ΔX₂ (mg)</th>
<th>ΣX₂ (mg)</th>
<th>(X₀₀¹₂ - X₂) (mg)</th>
<th>K (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-10</td>
<td>18.02</td>
<td>18.02</td>
<td>14.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>10.51</td>
<td>28.53</td>
<td>3.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>1.82</td>
<td>30.35</td>
<td>1.83</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>0.80</td>
<td>31.15</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>0.44</td>
<td>31.59</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>0.23</td>
<td>31.81</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80</td>
<td>0.36</td>
<td>32.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0-10</td>
<td>16.98</td>
<td>16.98</td>
<td>15.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>10.54</td>
<td>27.52</td>
<td>4.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>2.34</td>
<td>29.85</td>
<td>2.37</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>0.90</td>
<td>30.75</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>0.60</td>
<td>31.35</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>0.39</td>
<td>31.73</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80</td>
<td>0.48</td>
<td>32.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ X₀₀¹₂ = 32.18 mg cocaine for Experiment No. 1, 32.22 mg for Experiment No. 2.
TABLE 17

Estimation of the first-order release rate constant from a plot of the amount of cocaine in coca-chewing gum remaining to be released into water as test medium versus time.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Release Period (min)</th>
<th>$\Delta X_2$ (mg)</th>
<th>$\Sigma X_2$ (mg)</th>
<th>$(X_2^{10} - X_2)$ (mg)</th>
<th>Release Rate (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-10</td>
<td>9.84</td>
<td>9.84</td>
<td>5.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>0.27</td>
<td>10.12</td>
<td>0.28</td>
<td>$K_1 = 0.180$ (r=-0.941)</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>0.07</td>
<td>10.19</td>
<td>0.21</td>
<td>$K_2 = 0.046$ (r=-0.986)</td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>0.06</td>
<td>10.25</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>0.05</td>
<td>10.30</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>0.05</td>
<td>10.35</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80</td>
<td>0.05</td>
<td>10.40</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0-10</td>
<td>10.28</td>
<td>10.28</td>
<td>0.28</td>
<td>$K_1 = 0.211$ (r=-0.922)</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>0.12</td>
<td>10.40</td>
<td>0.15</td>
<td>$K_2 = 0.046$ (r=-0.984)</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>0.03</td>
<td>10.43</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-40</td>
<td>0.03</td>
<td>10.46</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>0.03</td>
<td>10.49</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>0.03</td>
<td>10.52</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-80</td>
<td>0.03</td>
<td>10.55</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

$^1 X_2^{10} = 10.40$ mg cocaine from Experiment No. 1, 10.55 mg for Experiment No. 2.
TABLE 18

Estimation of the first-order release rate constant, \( K \), from a plot of the amount of cocaine in coca-chewing gum remaining to be released into artificial saliva as test medium versus time.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Release Period (min)</th>
<th>( \Delta X_2 ) (mg)</th>
<th>( \Sigma X_2 ) (mg)</th>
<th>( (X_2^\infty - X_2) ) (mg)</th>
<th>( K ) (min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-10</td>
<td>13.35</td>
<td>13.35</td>
<td>0.55</td>
<td>0.302</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>0.52</td>
<td>13.87</td>
<td>0.03</td>
<td>( r=-0.999 )</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>0.03</td>
<td>13.90</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0-10</td>
<td>13.35</td>
<td>13.35</td>
<td>0.47</td>
<td>0.305</td>
</tr>
<tr>
<td></td>
<td>10-20</td>
<td>0.44</td>
<td>13.79</td>
<td>0.03</td>
<td>( r=-0.998 )</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>0.03</td>
<td>13.82</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

\(^1 \) \( X_2^\infty = 13.90 \text{ mg cocaine for Experiment No. 1}, \ 13.82 \text{ mg for Experiment No. 2.} \)
TABLE 19

Comparison of first-order release rate constants (K) for cocaine in cocaine- and coca-chewing gums studied in vitro in water and artificial saliva.

<table>
<thead>
<tr>
<th></th>
<th>First-Order Rate Constant (min⁻¹)</th>
<th>Water</th>
<th>Artificial Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. No. 1</td>
<td>Exp. No. 2</td>
<td>Exp. No. 1</td>
</tr>
<tr>
<td>Cocaine-Chewing Gum</td>
<td>K</td>
<td>0.079</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>K₁</td>
<td>0.180</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td>K₂</td>
<td>0.046</td>
<td>0.046</td>
</tr>
<tr>
<td>Coca-Chewing Gum</td>
<td>K</td>
<td>0.302</td>
<td>0.305</td>
</tr>
</tbody>
</table>
in artificial saliva was 0.304 min⁻¹. The release of cocaine from coca-chewing gum in water, however, showed two distinct phases: an immediate, rapid phase followed by a slower, more sustained phase. The immediate release rate constant, $K_1$, was calculated from the data for the first 30-min period, and the sustained release rate constant, $K_2$, was calculated for the remaining 40-80 min release period. A mean value of 0.196 min⁻¹ was obtained for $K_1$ and 0.046 min⁻¹ for $K_2$. 
Chapter V
DISCUSSION

5.1 COCAINE HYDROCHLORIDE

The melting point, UV and IR spectra of the cocaine hydrochloride used in the present research were found comparable to the literature values reported for the alkaloid. The UV spectra of cocaine hydrochloride dissolved in methanol showed absorption maximum at 230, 274 and 281 nm. Since the UV spectra of cocaine are similar to those given by cinnamoylcocaine, tropacocaine and benzoylecgonine (22, 81, 96), the UV method is neither specific nor sufficiently sensitive for the final identification of cocaine. IR spectrophotometry, however, is more useful for the identification of cocaine hydrochloride. The present cocaine hydrochloride had characteristic peaks at 1106, 1275 and 1728 cm$^{-1}$.

Cocaine potency can be easily assessed by the GC method of Moore (93) using Chromosorb W-HP, 80/100 mesh, as stationary phase coated with 3% OV-101. Moore's method, however, has a serious limitation in that it relies on an external standard in a separate GC tracing. In the present procedure n-PEBE, which has chemical and structural similarity to cocaine, was chosen as internal standard on the basis
of it having a suitable retention time relative to cocaine and an adequate GC peak response. Because cocaine is volatile at elevated temperatures, 0.6 N HCl in 2-propanol was added to samples containing free cocaine to prevent possible loss of cocaine during evaporation procedures. In the present GC assay, cocaine and n-PEBE on treatment with bis(trimethylsilyl)trifluoroacetamide (BSTFA), employed as derivatizing agent, do not undergo derivatization. The derivatization step, however, was employed as a means of differentiating cocaine from its hydrolysis products, namely: benzoylecgonine, ecgonine methyl ester and ecgonine. Normally, BSTFA replaces labile hydrogens in polar compounds with a Si(CH₃)₃ group yielding volatile and thermally stable trimethylsilyl-derivatives. The relative GC retention times for cocaine and the internal standard, n-PEBE, were 7.9 and 8.6 min, respectively. The standard concentration curve for cocaine was linear over the range of 0.4 to 1.6 µg of cocaine injected into the GC (r = 1.000, mean CV = 2.76%) indicating good precision and accuracy (Fig. 7). The minimal detectable quantity calculated from the standard curve for cocaine was 0.017 µg.
5.2 COCA LEAF POWDER AND COCA EXTRACTS

The genus *Erythroxylum* is the only natural source of cocaine and its related alkaloids. In the present study, an extraction/GC assay method was successfully employed for the quantitation of cocaine in *E. coca* and *E. novogranatense* var. *truxillense*. Cocaine is a base with a pKa of 8.6 and is best extracted at alkaline pH. Since the rate of cocaine hydrolysis becomes significant at a pH level higher than 9, relatively mild alkaline conditions were adopted for the extraction procedure. As recommended by Turner (79), sodium bicarbonate and 1.5% citric acid were used for the acid-base partitioning in the clean-up step. This combination gave nearly quantitative recovery of cocaine without undergoing hydrolysis. The recovery of cocaine from standards of increasing concentrations subjected to the procedure used to analyze the cocaine content of coca leaf powder was found to be 98.35% (r = 1.000) (Fig. 9).

The cocaine content of powdered leaves of *E. coca* and *E. novogranatense* var. *truxillense* available for the present studies as measured by the GC method was 0.44% and 0.48%, respectively. The cocaine concentration of the final 500 mL-ethanolic extract of *E. coca* and of *E. novogranatense* var. *truxillense* prepared by percolation of the leaf powders was 0.50% and 0.58%, respectively. The higher than expected cocaine content found in the coca leaf extracts may have resulted from utilizing a more efficient extraction procedure.
for the ethanolic extracts than for the powdered coca leaf analysis. In the procedure for the preparation of ethanolic coca extracts, percolation using boiling ethanol at the initial extraction stage is employed, whereas in the assay procedures for coca leaf powder only moderate heat (60°C) for 10 min is employed for the extraction of cocaine. The overall cocaine content of these preparations was comparable to published data (8, 79). The somewhat lower levels of cocaine in the coca leaves of the present study may also be attributed to some deterioration of the leaf materials during the relatively long time period between collection of the samples and when analysis was done in the laboratory.

In the chromatograms obtained in the GC analysis of the two coca leaf species (Fig. 8), small peaks suggestive of benzoylecgonine, ecgonine methyl ester, and ecgonine trimethylsilyl-derivatives were observed, but were too small to be measured. These cocaine-related compounds did not result from the hydrolysis of cocaine during its extraction since such peaks are absent in similar GC-chromatograms obtained in the cocaine recovery studies using cocaine standard solutions. These compounds found in the present coca leaf materials can be considered to be minor natural constituents of the leaves of the coca plant (21, 22).

GC fingerprinting provided a simple approach for differentiating between the overall composition of ethanolic extracts of E. coca and of E. novogranatense var. truxil-
lensense. According to the GC tracings, cocaine eluted with the same retention time of 13.1 min for both the derivatized (BSTFA) and non-derivatized samples of the two coca plants. As predictable, additional and higher peaks appeared in the chromatograms of derivatized samples compared with those of non-derivatized samples. Only the peak heights of cocaine were found to be comparable in the chromatograms of derivatized and non-derivatized samples. This finding again confirms that derivatization is not necessary for GC analysis of cocaine. The overall pattern of the gas chromatographic tracing of the derivatized (and also of the non-derivatized) samples of E. coca was similar to that of E. novogranatense var. truxillense. A considerable number of peaks with similar retention times are present in both tracings. The chromatogram obtained from the derivatized sample of E. coca, however, revealed three characteristic peaks appearing late in the tracing, which were absent in the tracing for the derivatized sample of E. novogranatense var. truxillense. The size of most of the peaks in the tracings of derivatized and non-derivatized sample of E. coca was greater than the size of corresponding peaks for E. novogranatense var. truxillense. These GC fingerprints were reproducible when examined for different batches of the ethanolic extracts of leaves from the two coca plants.
5.3 CHEWING GUM FORMULATIONS

As previously suggested, coca leaves because of their alkaloidal content may have some value for the prophylactic/therapeutic treatment of motion sickness. Coca chewing is a unique but an uncontrolled method of drug administration: initially highly active compounds diffuse quickly across the oral mucosa into the blood stream and later in a more sustained manner across the gastrointestinal tract. As a better approach for the experimental control of these absorption processes, chewing gum was formulated with cocaine and coca leaf extract to have well defined, uniform and reproducible release characteristics.

A three-step process was developed for formulating coca chewing gum: (a) preparation of a suitable ethanolic extract of dry coca leaf powder, (b) preparation of coca-dextrin by adsorption onto dextrin of the ethanol-soluble residue of coca leaf powder and (c) incorporation of the coca-dextrin into a chewing gum formulation with acceptable kneadability and consistency characteristics. Similarly, cocaine-chewing gum was formulated with cocaine hydrochloride as starting material.

The cocaine- and coca-chewing gum formulations were prepared with slight modification according to formulation No. 14 for cocaine-free chewing gum described in Table 2. In formulating the latter, corn syrup and water and/or 2%
methyl cellulose solution were added in that order to the softened gum base and mixed. To this was added dextrin and the mixture kneaded. The order (which was found to be critical) and the time of mixing (10 min) were kept constant for the formulation studies. The maximum percentage of dextrin for an acceptable chewing gum formulation was found to be no more than 52%. When a greater proportion of dextrin was incorporated into the formulation, kneading became difficult producing firm hard lumps. The gum base could be increased up to a maximum of 50% without resulting in a hard consistency. When the proportion of gum base was less than 15%, the gum became too soft and would disintegrate on chewing. When the liquid portion of the formulation (24%) was made up of water, the resulting gum also showed too soft a consistency, whereas substitution of the liquid portion with corn syrup made kneading difficult or impossible. Dividing the 24% liquid portion approximately equally between water (13%) and syrup (14%) resulted in the proper workable consistency.

The best workable formula for the cocaine- and coca-chewing gums consisted of 26% gum base, 13% corn syrup, 47% cocaine- or coca-dextrin, 13% water and 1% glycerin. At this composition, the kneadability and consistency of the cocaine-chewing gum was quite satisfactory, but the coca-chewing gum had a somewhat softer consistency. The lower consistency may be attributable to the properties of the coca
extract. Since the latter includes a wide range of different materials, it would be difficult to sort out the contributing factors without isolating and testing each one separately. The prepared formulations of cocaine- and coca-chewing gums on GC analysis showed a concentration of 1.08% and 0.45% cocaine, respectively. The more than 2-fold difference in cocaine concentration between the cocaine- and coca-chewing gums is due to the use of cocaine-dextrin formulated with a potency of 2.25% and of coca-dextrin with a potency of 0.98% cocaine.

Artificial saliva appeared to be a useful test medium for the in vitro release studies of cocaine from cocaine- and coca-chewing gums. The composition of the artificial saliva was chosen so as to duplicate the viscosity and electrolyte concentration of whole natural saliva. As expected, since cocaine breaks down into benzoylecgtonine and ecgontine primarily under basic conditions, cocaine for the most part remained unchanged during the release studies as performed in artificial saliva at pH 7.04 for 80 min at 37°C. As evident in the tracings from the GC assay shown in Fig. 12, peaks of egonine, egonine methyl ester and benzoylecgtonine occur but are negligible. The hydrolysis of cocaine in artificial saliva was minimal throughout the release study. That the conversion of cocaine to benzoylecgtonine is minimal at pH 7.04 has been previously reported (97).
There were significant differences in the release profiles of cocaine between water and artificial saliva. Cumulative percent of cocaine released from cocaine-chewing gum in water and in artificial saliva at the end of the 80-min test period was 34.6% and 89.2%, respectively. Overall release of cocaine in both water and artificial saliva was greater from coca-chewing gum than from cocaine-chewing gum. Cumulative percent of cocaine released from coca-chewing gum for the same 80-min test period in water and artificial saliva was 74.6% and 92.1%, respectively. Of the total cocaine released from the cocaine-chewing gum into water and artificial saliva, 80% and 87%, respectively, were released during the first 20 min. Whereas for coca-chewing gum, 97.9% of the total cocaine released into water was released in the same initial 20-min period, and 99.8% into artificial saliva. The higher initial release rate of cocaine from the coca-chewing gum was probably due either to its softer consistency over that of the cocaine-chewing gum or to some unique property of the coca extract.

The mean total recovery of cocaine (the sum of the amount found in both the test medium and remaining in the spent gum) from the release studies of cocaine-chewing gum in water and artificial saliva was 52.2% and 97.7%, respectively. Whereas for coca-chewing gum the recoveries in water and artificial saliva were 75.8% and 92.0%, respectively. Since the cocaine hydrolysis observed was negligible during
the release studies, the amount of cocaine not recovered is probably contained in the gum residue fragments left behind in the test medium.

For the present in vitro release studies, the "sigma-minus" method provided a simple direct approach for calculating the first-order release constant for cocaine in the several chewing gum formulations. The method, however, depends on having an accurate value for $X_t^2$, i.e., the total cumulative amount of cocaine ultimately released from the gum into the medium. For the present release experiments it was assumed that the total amount of cocaine released over the entire 80-min experimental period represents a reasonable estimate of $X_t^2$. The experimentally determined value was more than 95% of $X_t^2$.

For cocaine-chewing gum the release rate constants for cocaine in both water and artificial saliva were similar: 0.071 min$^{-1}$ and 0.073 min$^{-1}$, respectively. In both cases approximately 7.0% of the cocaine content was released per min. Release of cocaine from coca-chewing gum into artificial saliva also occurred at a uniform but at a much faster rate; the mean K value was 0.304 min$^{-1}$, representing a release rate of about 30% per min. In water, however, the release of cocaine from coca-chewing gum appeared to occur at two different rates: a fast initial release phase followed by a more sustained phase. The mean release rate constant ($K_1$) for the former was 0.196 min$^{-1}$ and for the latter ($K_2$)
The K₂ value is of the same order of magnitude as the overall K value for the several other gum formulations in the two test media. Thus, except for its initial rapid release, cocaine is released from the coca-chewing gum in a manner similar to the other formulations. The biphasic release rate pattern of coca-chewing gum in water is rather unique for which there is for now no obvious explanation.

Although the in vitro release profiles of cocaine from cocaine- and coca-chewing gums have been studied in both water and artificial saliva, it is difficult to predict from the results of the present studies the in vivo release profile of cocaine from these gum formulations. The in vitro test system used for the present release studies is deficient in a number of respects. The artificial saliva lacks mucins, enzymes (such as salivary amylase) and microorganisms capable of metabolizing drugs. Also, the present in vitro release studies were carried out under virtually static conditions in contrast to the highly dynamic conditions of the in vivo situation, corresponding to the natural act of mastication and swallowing involving the complex interaction of saliva, tongue, teeth, gums, oral mucosa and facial musculature. An in vivo release study in man will be necessary to provide the definitive release profile of cocaine from these chewing gum formulations. A further formulation study may be required to provide the optimum composition of coca-chewing gum formulation.
In a previous section, reference was made to the prophylactic/therapeutic potential of coca for the treatment of motion sickness. The antimotion sickness properties of cocaine or coca extract, however, remain to be critically tested. The present chewing gum formulation represents a suitable dosage form for the incorporation of cocaine or coca leaf extract which can be used under controlled conditions for their evaluation for antimotion sickness properties.
Chapter VI
SUMMARY AND CONCLUSIONS

Coca may be useful for the treatment of motion sickness since it has been used as a remedy for altitude sickness among South American Indians. Whether or not cocaine and/or coca leaf constituents produce relief from motion sickness, however, remains to be demonstrated. A novel oral dosage form containing either cocaine or coca leaf extract was designed and formulated to be used for possible evaluation for the treatment of motion sickness during space flight. Chewing gum was chosen as the most suitable dosage form since it would most closely duplicate coca leaf for chewing and possesses certain advantages over other conventional dosage forms when used under conditions of zero-G.

Melting point, IR and UV spectra were used to confirm the identity of the cocaine hydrochloride available for the present research. The determination of the cocaine potency of the several cocaine-containing preparations was successfully performed by a GC method using a Hewlett-Packard HP 5711 GC equipped with a flame ionization detector and n-PEBE as internal standard. The internal standard, n-PEBE, used in the present GC procedure showed a suitable retention time relative to cocaine and an adequate GC peak response. A der-
ivatization step was employed in the GC procedure as a means of differentiating cocaine from its hydrolysis products. The standard concentration curve for cocaine was linear over the useful range of 0.4 to 1.6 µg injected into the GC with a near zero-intercept. The assay had good precision and accuracy.

The cocaine content of powdered leaves of E. coca and of E. novogranatense var. truxillense was determined by extracting cocaine with 95% ethanol, acid-base partitioning with methylene chloride as a clean-up, and quantification by GC. Hydrolysis of cocaine was not observed during the assay of powdered coca leaf for cocaine content.

An ethanolic extract of two types of coca leaf was prepared by extraction followed by percolation. The cocaine content of powdered coca leaves and ethanolic extracts of E. coca and of E. novogranatense var. truxillense was comparable to published data.

GC fingerprinting was performed to provide a simple approach for differentiating between the overall composition of ethanolic extracts of E. coca and of E. novogranatense var. truxillense. Cocaine eluted with the same retention time in the GC tracing for both the derivatized and non-derivatized samples of the two coca plants. The overall pattern of the gas chromatographic tracing of the derivatized samples of E. coca was similar to that of E. novogranatense
var. *truxillense*. The chromatogram obtained from the derivatized sample of *E. coca*, however, revealed three characteristic peaks appearing late in the tracing, which were absent in the tracing for the derivatized sample of *E. novogranatense* var. *truxillense*. These fingerprints were reproducible when examined for different batches of the ethanolic extracts of leaves from the two coca plants, and served as confirmatory aids in the standardization of the two coca leaf extracts.

In order to insure a more uniform dispersion of coca alkaloids in the chewing gum formulation, the residue of the ethanolic extract of powdered leaves of *E. novogranatense* var. *truxillense* was first adsorbed onto dextrin producing coca-dextrin. Cocaine-dextrin was also prepared by adsorbing cocaine hydrochloride onto dextrin in a similar manner.

Chewing gum formulations containing cocaine-free dextrin were first prepared and examined to determine the gum composition offering optimum kneadability and consistency. The final formulation used to prepare cocaine- and coca-chewing gums is composed of 26% gum base, 13% corn syrup, 13% water, 1% glycerine, and 47% cocaine- or coca-dextrin. The kneadability and consistency of cocaine-chewing gum were satisfactory. The coca-chewing gum, however, showed a softer consistency which may be attributable to the unique properties of the coca extract. The cocaine content of chewing gums was determined by extraction of cocaine using a cold-
trituration process, acid-base partitioning followed by GC quantification.

The in vitro release studies were performed for cocaine- and coca-chewing gums in both water and artificial saliva at 37°C. Except for the lack of enzymes, artificial saliva closely approximated the composition of whole human saliva. The extraction efficiency or recovery of cocaine from artificial saliva examined was quantitative.

Of the total cocaine released, 80% was released from cocaine-chewing gum in both water and artificial saliva in the first 20-min of the 80-min test period, whereas 80-90% from coca-chewing gum. Overall total release of cocaine from coca-chewing gum was similar in both water (ca. 75%) and artificial saliva (ca. 90%). Cocaine-chewing gum also exhibited the same overall total release (ca. 90%) in artificial saliva, but was much lower in water (ca. 35%). The release profiles of cocaine from all chewing gum formulations studied in both water and artificial saliva were reproducible.

Pharmacokinetic analysis of the data obtained from the present in vitro release studies of cocaine- and coca-chewing gums suggests some significant quantitative differences between the two types of formulations. Compared to the coca-chewing gum, the release behavior of the cocaine-chewing gum was more consistent; in both water and artificial saliva cocaine was released uniformly at a rate of approximately 7%
per min over the 80-min period. The release behavior of coca-chewing gum was considerably more variable in both water and artificial saliva. Release of cocaine from coca-chewing gum proceeded initially at a much faster rate ($K = 0.304 \text{ min}^{-1}$ for artificial saliva and $0.191 \text{ min}^{-1}$ for water). Furthermore, in water release of cocaine occurs in two distinct phases: approximately 20% per min for the first 30 min and 5% per minute for the remaining 50 minutes. In terms of the rate and extent of drug delivery as determined in the present in vitro release studies, it may be concluded that the cocaine-chewing gum as an oral dosage form of cocaine is a more dependable, efficient formulation than coca-chewing gum. It is difficult, however, to predict from the results of these in vitro studies the in vivo release profile of cocaine from these gum formulations. An in vivo release study conducted in man would be necessary to provide the definitive release profile of cocaine from these formulations.
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