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Preparation of Codeine-Resinate and Chlorpheniramine-Resinate Sustained-Release Suspension and its Pharmacokinetic Evaluation in Beagle Dogs

Huan-Xiang Zeng, Gang Cheng and Wei-San Pan
Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang, P. R. China

Guo-Ping Zhong and Min Huang
Faculty of Pharmacy, Sun Yat-sen University, Guangzhou, P. R. China

Address correspondence to Huan-Xiang Zeng, Department of Pharmaceutics, Shenyang Pharmaceutical University, No. 103, Wenhua Road, Shenyang, 110016, the People's Republic of China; E-mail: zenghxg@yahoo.com

ABSTRACT Using ion exchange resins (IERs) as carriers, a dual-drug sustained release suspension containing codeine, and chlorpheniramine had been prepared to elevate drug safety, effectiveness and conformance. The codeine resinate and chlorpheniramine resinate beads were prepared by a batch process and then impregnated with Polyethylene glycol 4000 (PEG 4000), respectively. The PEG impregnated drug resinate beads were coated with ethylcellulose as the coating polymer and di-n-butyl-phthalate as plasticizer in ethanol and methylene chloride mixture by the Wurster process. The coated PEG impregnated drug resinate beads were dispersed in an aqueous suspending vehicle containing 0.5% w/w xanthan gum and 0.5% w/w of hydroxypropylmethylcellulose of nominal viscosity of 4000 cps, obtaining codeine resinate and chlorpheniramine resinate sustained-release suspension (CCSS).

Codeine phosphate and chlorpheniramine maleate were respectively loaded onto AMBERLITE® IRP 69, and PEG 4000 was used to impregnate drug resinate beads to maintain their geometry. Ethylcellulose with di-n-butylphthalate in ethanol and methylene chloride mixture for the coating of drug resinate beads was performed in Glatt fluidized bed coater, where the coating solution flow rate was 8–12 g/min, the inlet air temperature was 50–60°C, the outlet air temperature was 32–38°C, the atomizing air pressure was 2.0 bar and the fluidized air pressure was adjusted as required. Few significant agglomeratation of circulating drug resinate beads was observed during the operation. The film weight gained 20% w/w and 15% w/w were suitable for the PEG impregnated codeine resinate and chlorpheniramine resinate beads, respectively. Residual solvent content increased with coating level, but inprocess drying could reduce residual solvent content.

In the present study, the rates of drug release from both drug resinate beads were measured in 0.05*M* and 0.5*M* KCl solutions. The increased ionic strength generally accelerated the release rate of both drugs. But the release of

codeine from its resinate beads was much more rapid than chloropheneramine released from its resinate beads in the same ionic strength release medium. The drug release specification of the CCSS, where release mediums were 0.05*M* KCl solution for codeine and 0.5*M* KCl solution for chlorpheniramine, was established to be in conformance with in vivo performance.

Relative bioavailability and pharmacokinetics evaluation of the CCSS, using commercial immediate-release tablets as the reference preparation, were performed following a randomized two-way crossover design in beagle dogs. The drug concentrations in plasma were measured by a validated LC-MS/MS method to determine the pharmacokinetic parameters of CCSS. This LC-MS/MS method demonstrated high accuracy and precision for bioanalysis, and was proved quick and reliable for the pharmacokinetic studies. The results showed that the CCSS had the longer value of T_{max} and the lower value of C_{max}, which meant an obviously sustained release effect, and its relative bioavailability of codeine and chlorpheniramine were (103.6 ± 14.6)% and (98.1 ± 10.3)%, respectively, compared with the reference preparation. These findings indicated that a novel liquid sustained release suspension made by using IERs as carriers and subsequent fluidized bed coating might provide a constant plasma level of the active pharmaceutical ingredient being highly beneficial for various therapeutic reasons.

KEYWORDS Codeine, Chlorpheniramine, Sustained release, Oral suspension, Ion exchange resins, Coating, Wurster process, Pharmacokinetics

INTRODUCTION

Drug delivery systems (DDSs) are flourishing due to in depth study of administration theory and the development of polymer material science. More and more polymeric excipients have been studied in order to make drug release fit for the desired profiles. Among them, ion exchange resins (IERs), which are insoluble polymers that contain acidic or basic functional groups and have the ability to exchange counter ions with aqueous solutions surrounding them, have also received considerable attention from pharmaceutical scientists because of their versatile properties as drug delivery vehicles. Much of research over the past few years have revealed that IERs are very suitable for drug delivery technologies, including sustained, controlled, rapid, site-specific, and taste-masked release systems (Vikas et al., 2001; Hughes, 2004).

In practice, drug in an ionic form is mixed with the appropriate IERs to form a complex, known as resinate. Drug release from resinate relies on the ionic environment and should therefore be less susceptible to other conditions, such as enzyme content, at the site of absorption. Therefore, many formulation studies for peroral DDSs based on IERs have been reported recently, and IERs have imparted flexibility in designing peroral DDSs, such as complexes (Agarwal et al., 2000; Akkaramongkolporn, 2001, 2006), microcapsules (Sriwongjanya & Bodmeier, 1997; Sprockel & Price, 1989; Fundueanu et al., 2005), floating systems (Atyabi et al., 1996; Kouchak & Atyabi, 2004) and liquid suspensions (Raghunathan, 1980; Sprockel & Price, 1989; Pongpaibul et al., 1990; Cuna et al., 2000).

There are many cases, especially for pediatric or elderly patients, where an oral suspension is a favorable dosage form because of the ease in swallowing liquid and the flexibility in adjusting the dose. A dual-drug sustained release suspension containing codeine and chlorpheniramine would be one of the desirable dosage forms, since both drugs are often used in the elderly patients for treatment of chronic diseases such as cough and allergy. But the major drawback of common sustained release systems is dose dumping, resulting in increased risk of toxicity. IERs have drug retaining properties and prevention of dose dumping, because of the physical and chemical properties. Moreover, IERs can prevent the diffusion of drug when suspended in a nonionic medium, because drug release will be only promoted by the presence of competing ions, such as occurs in the gastrointestinal tract upon oral administration. The drug release rate from drug resinate beads can be further controlled by coating the drug-resinate beads using a variety of microencapsulation or coating processes (Motycka & Nairn, 1979; Motycka et al., 1985; Moldenhauer & Nairn, 1990; Torres et al., 1995).

Codeine phosphate is chemically 7,8-didehydro-4,5-α-epoxy-3-methoxy-17-methyl-morphinan- 6-α-ol phosphate salt. In pharmaceutical preparations, it is used as a sedative, an analgesic and an antitussive

agent (Sweetman, 2005; Thomson, 2006). Chlorpheniramine maleate is chemically 2-pyridinepropanamine, γ-(4-chlorophenyl)-*N*,*N*-dimethyl, (*Z*)-2-butenedioate maleate salt. It is an antihistamine and is widely used as an ingredient in antitussive formulations (Sweetman, 2005; Thomson, 2006). Codeine phosphate and chlorpheniramine maleate have been selected as model water soluble cationic drugs. Moreover, these two drugs are the pharmacologically active constituents found in most conventional cough-cold pharmaceutical preparations and completely absorbed following oral administration, appear to be suitable drugs candidate for liquid sustained release formulations because of their short biological half-lives and frequent administration. Codeine can produce drug dependence and, therefore, has the potential for abuse, while its compound formulation containing chlorpheniramine will prevent or reduce chances of abuse. At the same time, IERs can also be used to make it more difficult or less desirable to abuse such formulations (Hughes, 2004).

In the present study, preparation of codeine and chlorpheniramine resinate sustained release suspension (CCSS) and its pharmacokinetic evaluations in beagle dogs were performed. The sustained release properties could not be achieved by the drug resinates alone, so the coating of drug resinate beads was necessary. The absorption of the drug from coated drug resinates is a consequence of the entry of the counter ions into the coated drug resinates, release of drug ions from the drug resinates by the ion exchange process, and diffusion of drug ions through the semipermeable membrane into the surrounding absorption environment (Vikas et al., 2001).

In order to apply the coated drug resinate beads to a sustained release suspension, it was required that the size of the coated drug resinate beads had to be small enough to be easily suspended in an aqueous vehicle. However, the coating process of fine drug-resinate beads was often hampered by severe agglomeration depending on the physicochemical properties of the drug resinate beads and the coating spray system. In addition, the drug resinate beads could swell greatly when put in water and shrink when redried. This swelling may result in rupture of the coating film when the coated drug-resinate beads are suspended in an aqueous vehicle, destroying any sustained-release effects due to the rate controlling film (Hall, 1979).

Therefore, the present work is aimed at studying the coating process of drug resinate beads and the sustained release effect of the coated drug resinate beads in aqueous suspension. The drug resinate beads were formed by a bath process, a specific quantity of the IER was agitated with the drug solution until the equilibrium was established, and coated by the fluidized bed process subsequently, and then suspended in an aqueous vehicle of adequate viscosity and palatability. The formulations of CCSS were characterized, and the pharmacokinetics evaluation of CCSS, using commercial immediate-release tablets as the reference preparation, was performed in beagle dogs. The novel dual-drug sustained release suspension containing codeine and chlorpheniramine for oral administration made based on IERs as carriers would be of great help for cough and allergy patients, specially for children and older persons, who have difficulties in swallowing.

MATERIALS AND METHODS

Codeine phosphate was purchased from Qinghai Pharmaceutical Corporation (China). Chlorpheniramine maleate was purchased from Guangdong Pharmaceutical Industry Corporation (China). Loratadine was from National Institute for the Control of Pharmaceutical and Biological Products (China). Ion exchange resin (AMBERLITE® IRP 69) was from Rohm & Haas Shanghai Corporation (China). Ethylcellulose (ETHOCEL®, Standard 10 Premium, Dow Chemical Corporation, USA) and Hydroxypropyl methylcellulose (METHOCEL®, K4M Premium, Dow Chemical Corporation, USA) were kindly supplied by Colorcon Shanghai Corporation (China). Polyethylene glycol 4000 (PEG 4000) was purchased from Sinopharm Chemical Reagent Corporation (China). Polysorbate 80 was from Aldrich Chemical Company Inc. (USA). Di-n-butyl-phthalate, methyl 4-hydroxybenzoate, n-propyl 4-hydroxybenzoate, 1,2-propanediol and glycerol were obtained from ABCR GmbH & Co. KG (Germany). Sucrose was obtained from Dongguan Sugarhouse Corporation (China). Xanthan gum was from Shandong Deosen Corporation (China). Colors and flavors were obtained as gifts from Sensient Technologies Corparation (China). Acetonitrile, methanol, phosphoric acid, formic acid and ethyl acetate were purchased from Tedia Company Inc. (USA). Purification of water (purified water) was carried out by deionization and distillation. All the other chemicals and reagents used were of the analytical grade obtained commercially.

Fluidized bed coater (Model GCCP1.1, Glatt GmbH, Germany), liquid mixer (Model MAZELA Z, Tokyo Eyela Corporation, Japan), aspirator (Model A-3S, Tokyo Eyela Corporation, Japan), vacuum drier (Model VOS-451SD, Tokyo Eyela Corporation, Japan), stand mixer (Model KM800, Kenwood Appliance Corporation, England), analytical balance (Model XT220A, Precisa Instruments Ltd., Switzerland), magnetic stirrer (Model RCN-3D, Tokyo Eyela Corporation, Japan), dissolution apparatus (Model ZRS-8, Tianjing University Precision Corporation, China), high- performance liquid chromatograph (HPLC, Model Agilent 1100 series, USA), HPLC columns (Ph-3, 5 μm, 4.6×250 mm, GL Science Corporation, Japan), quantum mass spectrometer system equipped with surveyor MS pump and autosampler (Model TSQ, Thermo Electron Corporation, USA) were used in various experiments.

Drug-resinate Beads Preparation

The codeine and chlorpheniramine resinate beads were prepared by a batch process, respectively. AMBERLITE® IRP 69, a golden brown fine powder with a particle size of 45–125 μm, is a cation exchange resin prepared as the sodium form of the sulfonated styrene divinylbenzene copolymer. It is insoluble in water, the water content determined by Karl Fischer titrimetry is 6.8%, and its tapped density is 0.86 g/cm^3. Specified amount (420 g) of codeine phosphate or chlorpheniramine maleate was accurately weighed and put in 10 L stainless steel vessel with 6 L of purified water, respectively. Specified amount (800 g) of AMBERLITE® IRP 69 resin was added and suspended when the active pharmaceutical ingredient was dissolved under stirring at room temperature and then continuously stirring for 4 hr. The drug-resinate beads were recovered by precipitation or vacuum filtration and resuspended in 6 L of purified water. The suspension was stirred for 30 min at room temperature and then decanted. The washing process, removing the free drugs, was repeated until the drug concentration in the supernatant became negligible (below 10 μg/mL for codeine and 4 μg/mL for chlorpheniramine maleate). The recovered drug resinate beads were dried in a vacuum drier at 50°C to a constant weight, and then screened through a 100 mesh sieve to obtain the dry drug resinate beads. The percentage of both codeine and chlorpheniramine fixed to AMBERLITE® IRP 69 resin beads were 24.6% and 26.4%, respectively. The results of drug release from the codeine resinate beads and chlorpheniramine resinate beads are shown in Fig. 1.

PEG-impregnation of Drug-resinates

PEG-impregnated drug-resinates were prepared as follows, from a formulation of purified water (500 g), PEG 4000 (200 g), and codeine or chlorpheniramine

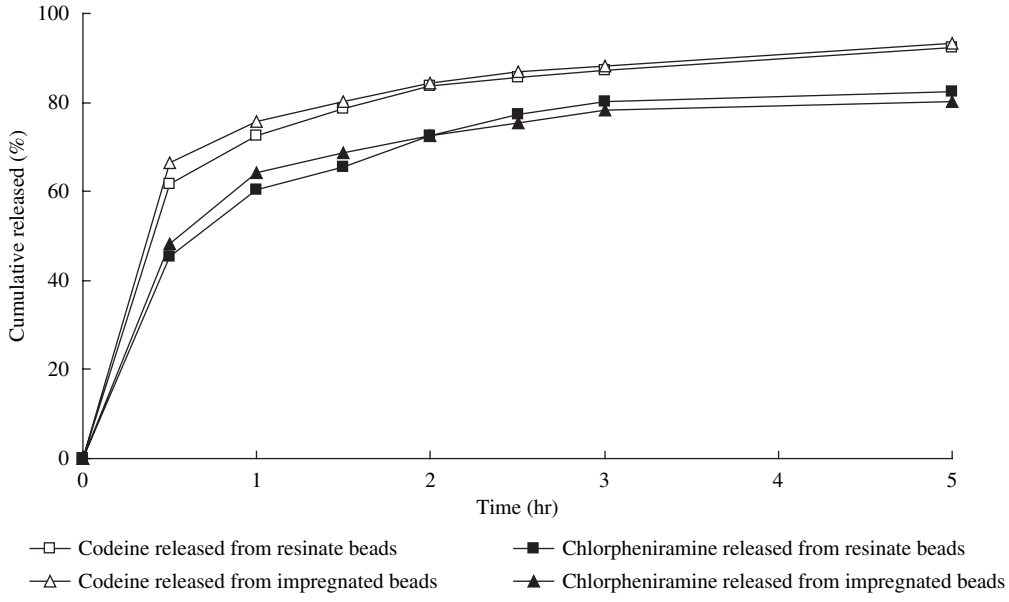

FIGURE 1 **Drug Release Profiles of Drug Resinate Beads and PEG-Impregnated Drug Resinate Beads.**

resinate beads (1000 g). The PEG 4000 was dissolved in the purified water and added slowly to a stand mixer containing codeine or chlorpheniramine resinate beads, respectively. After mixing for 30 min, the PEG-impregnated drug resinate beads were transferred to a vacuum drier and dried at 50°C, and the drying process was performed for about 10 hr to reduce the water content of the PEG-impregnated drug resinate beads to less than 8% w/w. The PEG-impregnated drug resinate beads were fractionated by sieving. The profiles of drug release from the PEG-impregnated drug resinate beads are shown in Fig. 1.

Coating Process

Using the Wurster process techniques, the PEG-impregnated drug resinate beads were coated with ETHOCEL® ethylcellulose to different thickness. For coating, specified amount (600 g) of PEG-impregnated drug resinate beads with a particle size of 75–150 μm were used. Samples of 30 g coated PEG-impregnated drug resinate beads were removed from the fluidized bed coater when the coating weight gain of 5, 10, 15, or 20% was achieved on the PEG-impregnated drug resinate beads, respectively. The composition of the coating solution is shown in Table 1. A solution of ETHOCEL® ethylcellulose in ethanol and methylene chloride mixture was prepared by dispersing the 120 g of ETHOCEL® ethylcellulose powder in 1500 g of ethanol and methylene chloride mixture and allowing it to stand for 24 hr. Di-n-butyl-phthalate, as plasticizer, was also added. The final coating solution was agitated continuously throughout the coating processes. All the coating processes were performed using an fluidized bed coater (Wurster System) under the coating conditions shown in Table 1. At the conclusion of coating, the coated PEG-impregnated drug resinate beads were dried at current inlet heat and fluidization conditions with reduced atomization air to avoid bead attrition. The coated PEG-impregnated drug resinate beads were fractionated by sieving.

TABLE 1 **Formulations and Operating Conditions of Fluidized Bed Coating Process**

Core: PEG-impregnated drug-resinate beads	600 g
Coating formulation:	
ETHOCEL® ethylcellulose	120 g
Di-n-butyl-phthalate	30 g
Ethanol	300 g
methylene chloride	1200 g
Operating conditions:	
Inlet air temperature	50–60°C
Outlet air temperature	32–38°C
Liquid flow rate	8–12 g/min
Spray nozzle diameter	0.8 mm
Atomization air pressure	2.0 bar

The fluidized bed coater must be cleaned to avoid cross contamination with drug resinate beads during transfer to another one. Drug release results of the coated PEG-impregnated drug resinate beads are shown in Fig. 2. Those coated PEG-impregnated codeine resinate beads weight gained 20% w/w and coated PEG-impregnated chlorpheniramine resinates weight gained 15% w/w on the base of uncoated PEG-impregnated drug-resinate beads were found to contain 17.1% of codeine base and 27.2% of chlorpheniramine salt, respectively.

Residual Organic Solvents

Residual organic solvents of the coating film were determined by static headspace capillary gas chromatography on a Gas Chromatograph (GC 17A, Shimadzu, Japan) equipped with a flame ionization detector and Leap CombiPal headspace autosampler. Instrument control and data reduction was performed with Class VP software (Shimadzu, Japan).

Preparation of CCSS

The CCSS was prepared by adequately dispersing 70.2 g of coated PEG-impregnated codeine resinate beads and 8.8 g of coated PEG-impregnated chlorpheniramine resinate beads in 3 L of an aqueous suspending vehicle. Specified amount of xanthan gum, hydroxypropyl methylcellulose, sucrose, glycerol, polysorbate 80, preservative, colors and flavors, and other formulated excipients were accurately weighed and put in 5 L stainless steel vessel with 3 L of purified water to prepare the suspending vehicle under stirring. Specified amount of the coated PEG-impregnated codeine resinate beads was accurately weighed and added under stirring, and then continuously stirring for 10 min to obtain the CCSS. Each 5 mL of the CCSS contained 20 mg of codeine and 4 mg of chlorpheniramine maleate for codeine-resinate and chlorpheniramine-resinate, respectively. The CCSS samples

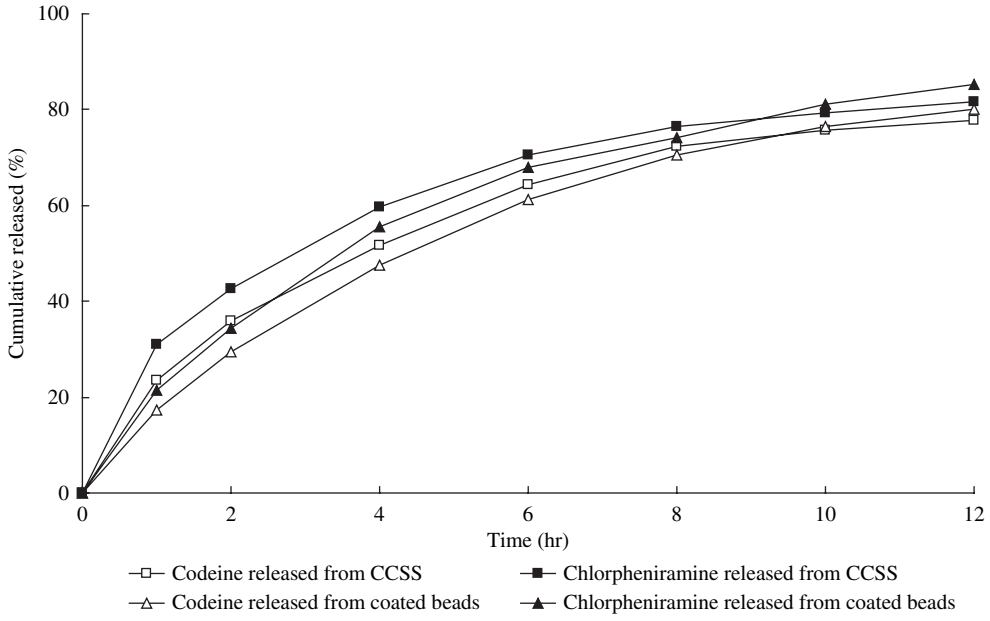

FIGURE 2 **Drug Release Profiles of Coated PEG-Impregnated Drug Resinate Beads and CCSS.**

were stored in closed glass vessels at 20°C and ambient humidity conditions, for a 6-month period.

Morphology and Bead Size Distribution

The morphology of the uncoated and coated PEG-impregnated drug resinate beads was examined by a thermal field emission environmental scanning electron microscopy (SEM) (Quanta 400 model, FEI Company, Oregon). Samples were mounted onto stubs using double sided adhesive tape and vacuum coated with gold film using a sputter coater (Edward S150, Watford, UK).

The bead size distribution of the uncoated and coated PEG-impregnated drug resinate beads was measured by a nest of Chinese Pharmacopoeia Standard sieves. Samples were placed on the top of the nest of Chinese Pharmacopoeia Standard sieves stacked from bottom to top in ascending order of aperture sizes ranging from 45–250 μm. The sieves were shaken using a mechanical shaker for 10 min. The beads that retained on each sieve were weighed and two replicates were performed for each batch of samples.

Drug Assay by HPLC

The amount of the active pharmaceutical ingredients in all kinds of drug resinate beads and CCSS was determined via HPLC analysis. CCSS was shaked well and aliquot of 10.0 mL was dissolved in 90.0 mL of admixture solvent (0.5*M* KCl:methanol=50:50 v/v) under magnetic stirring at 500 rpm for 18 hr. Thereafter, appropriate quantity of purified water was added to make into 100 mL, and then aliquot was filtered through a 0.45 μm membrane filter and 10 μL of the filtrate was directly injected for HPLC analysis. Separation was achieved by using column (Ph-3, 5μm, 4.6×250 mm, GL Science Corporation, Japan) at 30°C. Elution was performed as follows: flow rate 1.0 mL/min, 0–20 min, eluent acetonitrile/phosphate buffer (potassium dihydrogen phosphate 6.8 g, sodium 1-hexanesulfonate 0.5 g, triethylamine hydrochloride 0.5 g made into 1 L, pH 2.5) (20:80). The both active pharmaceutical ingredients were detected by absorbance at 220 nm with UV detector. Peak areas were directly proportional to mass of standards injected. The drug concentrations were determined by interpolation from a standard curve. The drug assay of all kinds of drug-resinate beads was performed as described above. That is, the drug resinate beads were shaken with admixture solvent,which was then analyzed by HPLC.

In Vitro Release Studies

The drug release profiles of all kinds of drug resinate beads and the CCSS were measured by the paddle method according to the Chinese Pharmacopeia

2005 edition. Specified amount of drug resinate beads or CCSS was placed in the dissolution apparatus filled with 900 mL of 0.05*M* KCl (for codeine) or 0.5*M* KCl (for chlorpheniramine). Release studies were performed at 37°C with an agitation of 75 rpm. Aliquot (5 mL) was withdrawn from the release medium at predetermined time points and the amount of released active pharmaceutical ingredients was determined via HPLC analysis as described above.

In Vivo Pharmacokinetics Studies

The in vivo study was conducted following a randomized two-way crossover design. A washout period of a week was allowed between two study phases. The reference products were codeine phosphate tablets (30 mg) made by Qinghai Pharmaceutical Corporation (China) and chlorpheniramine maleate tablets (4 mg) made by Hualong Pharmaceutical Corporation (China). Eight healthy adult beagle dogs (4 females, 4 males) with a mean body weight of 9.5 ± 0.4 kg purchased from Guangdong National Beagles Resources Research Center were used in this study. In order to validate the analytical methodology, determine the sampling schedule and assess variability, the pilot study in two beagle dogs had been carried out before proceeding of the crossover study.

Eight healthy adult beagle dogs were given serial numbers respectively and divided into two groups (4 in each group). After a fasting period of 12 hr, a single dose of the test products or the reference products (corresponding to the amount of 20 mg codeine and 4 mg chlorpheniramine maleate) was given orally at 8 a.m. to each subject. After a week washout period, the crossover study was performed. The eight subjects were given standard meals during the study phase. Blood samples (3 mL) were collected from the foreleg vein at before (baseline) and 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 9, 12, 24 hr after drug administration. Then, the heparinized blood samples were centrifuged at 4000 rpm for 10 min, and the plasma was stored at -30°C until assayed.

Bioanalysis of Plasma Sample by LC-MS/MS

The quantifications of codeine and chlorpheniramine in beagle plasma were performed by LC-MS/MS method using loratadine as the internal standard. The internal standard solution was prepared by dissolving 20.0 mg of loratadine in 100.0 mL 50% v/v methanol. The drugs and/or their metabolites in biological materials were extracted using liquid–liquid extraction technique. Fifty μL of the internal standard solution (0.2 ng/μL) was added to the plasma (500 μL). For basification, 50 μL of 0.1*M* NaOH solution was added to each sample followed by extraction with 4 mL of ethyl acetate for 5 min. After centrifugation (4000 rpm, 10 min), 2 mL of the organic phase was withdrawn and evaporated at 40°C under vacuum. The eluent (200 μL) was added to dissolve the residue and then centrifuged at 15,000 rpm for 3 min, aliquot (5 μL) of the supernatant fluid was injected for LC-MS/MS analysis.

The conditions of LC-MS/MS analysis are listed in Table 2. The mobile phases were methanol/water(80/20,v/v)solution with 0.1% v/v formic acid. The instrument was calibrated before sample analysis. Product ion scan of analyte, codeine and chlorpheniramine, and internal standard, loratadine, were carried out. The specific precursor ions and product ions were chosen based on these results. Instrument optimization was performed to achieve more sensitive detection base on these ion pairs. Codeine, chlorpheniramine and loratadine became $[M+H]^+$ molecular ion peaks under electrospray ionization (ESI) and were identified as three characteristic parent ions, m/z 300.07, $[M+H]^+$ of codeine, m/z 275.36, $[M+H]^+$ of chlorpheniramine and m/z 383.04, $[M+H]^+$ of loratadine, as product ions of m/z 152.00, 230.25 and 266.94, respectively. The retention time of codeine, chlorpheniramine and loratadine was about 0.76, 0.80 and 1.33 min, respectively. The total ion chromatogram (TIC) profiles of blank plasma and sample plasma showed that all the analyte peaks were well separated and free from interfering endogenous plasma constituents. The representative MS spectra and TIC profiles of codeine, chlorpheniramine and loratadine are shown in Figs. 3 and 4.

RESULTS AND DISCUSSION

Drug resinate beads can form when drugs in ionic forms are mixed with appropriate IERs in aqueous solution. Drugs can be loaded onto the reains by a batch process or a column process. The formation process of drug resinate beads is an ion exchange

TABLE 2 The Conditions of LC-MS/MS Analysis

Analytical column	Hypurity C_{18}, 5 μm
Flow rate (μL/min)	200
Injection volume (μL)	5
Tray temp (°C)	16
Column oven temp (°C)	30
Ionization source	ESI
Polarity	Positive
Spray voltage (V)	3500
Capillary temperature (°C)	350
Sheath gas pressure (psi)	30
Aux gas pressure (psi)	10
Run time (min)	2
Chrom filter peak width (m/z)	50
Scan width (m/z)	0.002
scan time (s)	0.1

Name	Codeine	Chlorpheniramine	Loratadine
Parent (m/z)	300.07	275.36	383.04
Product (m/z)	152.00	230.25	266.94
Tube lens offset	128	56	95
Source CID (V)	22	5	22
Collision pressure (mTorr)	1.5	1.5	1.5
Collision energy (V)	52	21	41
Q1 PW	0.2	0.5	0.5

equilibrium reaction. Therefore, the drug ions and competing ions can be exchanged in aqueous phase. This property allows drugs to be loaded onto resins and then released in vivo by ions present in gastrointestinal fluids.

The drug resinate beads possess physical properties similar to the IERs. Although it has been demonstrated that the release of drugs can be retarded by forming drug resinate beads, the need to further retard the release of certain drugs is demonstrated in Fig. 1. The rate of drug release from resinate beads depends on the polymeric and ionic properties of IERs, and it is also affected by salt concentration in release medium, the selectivity and diffusion rate of the drug (Vikas et al., 2001; Hughes, 2004).

AMBERLITE® IRP 69 resin beads, a gel type material, can swell greatly when placed in water and shrink when redried. In order to prepare a dual-drug sustained release suspension containing Codeine and chlorpheniramine, it was necessary that the drug resinate beads were treated, prior to coating, with a solvating agent such as polyethylene glycol (PEG), the resulting coated drug resinate beads retained their shape integrality, thus avoiding the further problem of rupture of the polymer coating (Hall, 1979). PEG 4000 was selected as having the desired properties, and PEG-impregnation alone did not retard the drug release from the resinate beads, however, the combination of PEG 4000 followed by an ethylcellulose overcoat had a greater effect. As shown in Figures 1 and 2, simple drug resinate beads and PEG-impregnated drug resinate beads released rapidly its drug, but the release rate was drastically retarded when the PEG-impregnated drug resinate beads were coated with ethylcellulose coating.

Coating Process

PEG-impregnated drug resinate beads were coated at levels of 5, 10, 15 and 20% by weight as combined weight gain of the ethylcellulose and plasticizer in the coated PEG-impregnated drug resinate beads, respectively. The coating solution was prepared by dissolving the plasticizer and ethylcellulose in ethanol and methylene chloride mixture. The coating of the PEG-impregnated beads was carried out in a fluidized bed coater (Wurster process) at a rate of 8–12 g/min of coating solution. The inlet and outlet air temperatures were about 50–60°C and 32–38°C, respectively. The atomizing air pressure was 2.0 bar and the fluidized air pressure was adjusted as required.

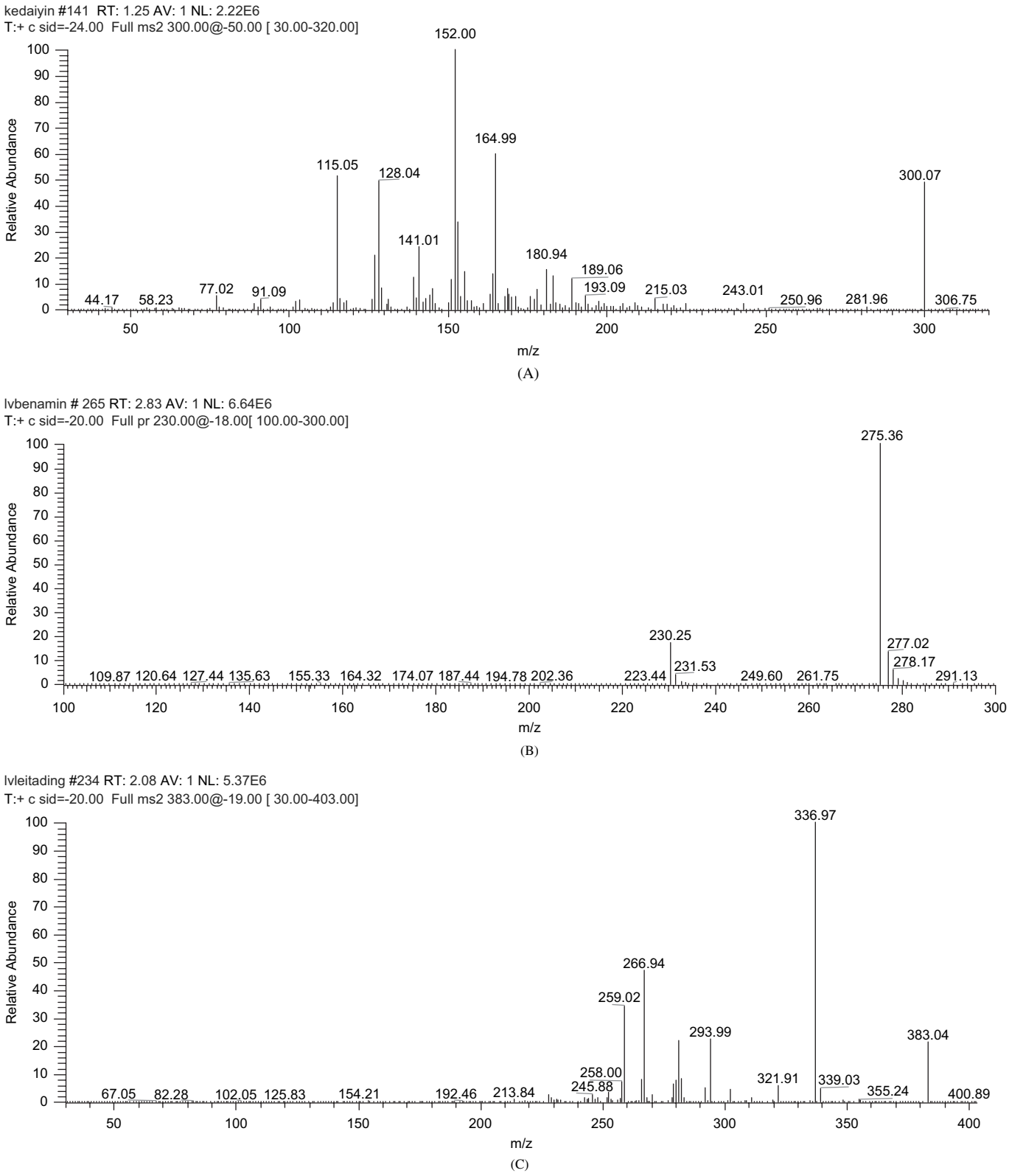

FIGURE 3 **MS Spectra of Codeine (A), Chlorpheniramine (B) and Loratadine (C).**

Ethylcellulose is a water insoluble polymer that has been used successfully for film coating applications in DDSs. Due to its high glass transition temperature (approximately 129–133°C), ethylcellulose has to be plasticized to improve its thermal behavior and tensile properties. Ethylcellulose films without plasticizer are

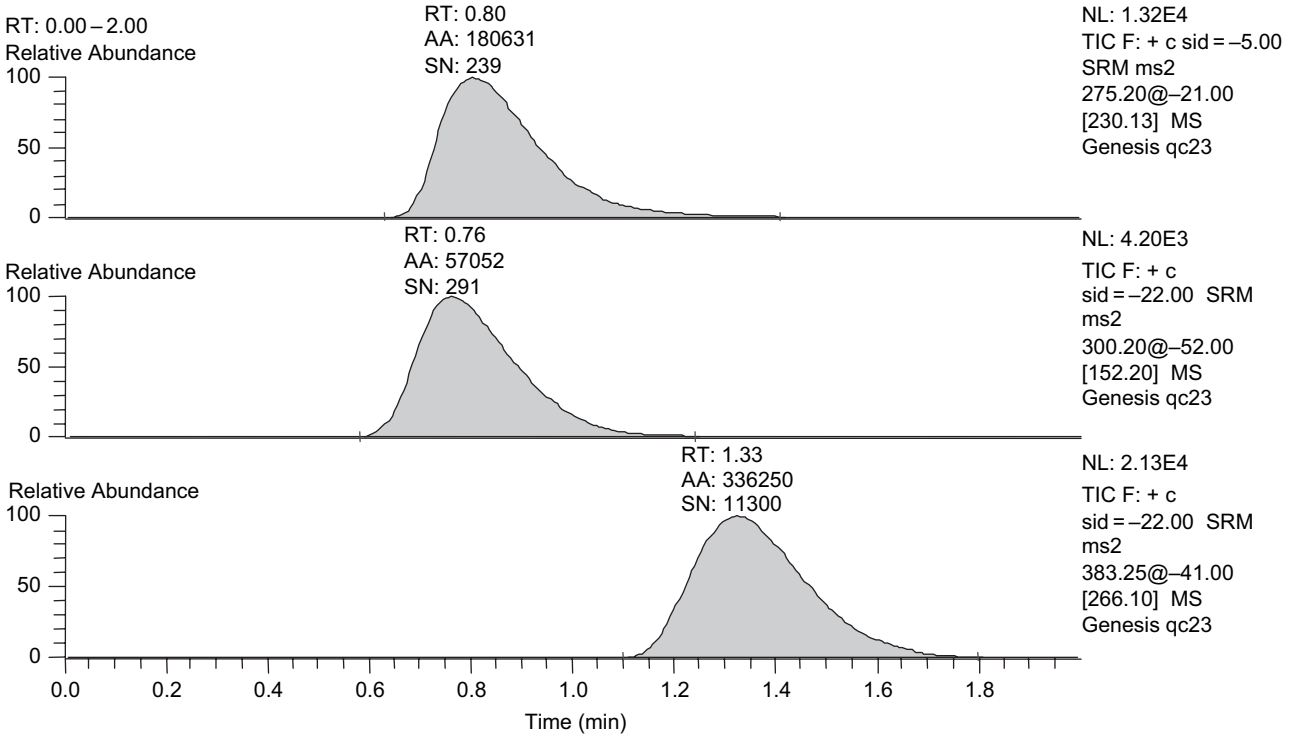

FIGURE 4 **TIC Profiles of Sample Plasma of Beagle Dogs After Peroral Single Dosing. Chlorpheniramine (Top), Codeine (Middle) and Loratadine (Internal Standard, Bottom).**

brittle and have low integrity. Adding small amounts of plasticizers can increase the flexibility and thus the integrity of ethylcellulose films. Several studies have shown the importance of the type and amount of the plasticizer and solvent used (Jones & Medlicott, 1995; Hyppola et al., 1996).

In the current study, a solution of ETHOCEL® ethylcellulose in ethanol and methylene chloride mixture was used. From the practical point of view, water based polymeric systems are preferable in development of coated pharmaceutical dosage forms to organic solvent based polymeric systems because of their environmental and economic disadvantages. But the drug resinate beads may swell during an aqueous coating process, and shrink on drying, causing the coating film to crack (Hall, 1979; Raghunathan, 1980). Additionally, film failure may be due to the migration of the highly water soluble codeine phosphate and chlorpheniramine maleate into the film coating. Therefore, the organic solvent based coating was used.

For ethtylcellulose, ethanol is a poor solvent and methylene chloride is a good solvent. The coating film made from a good solvent will be homogeneous and dense, but the high affinity of preferred solvents for the ethylcellulose may result in the tendency to agglomerate drug resinate beads during the coating process due to the higher viscosity of the solution. The extended ethylcellulose chains in a good solvent increase the viscosity of the solution due to the breaking of intramolecular association and chain-stiffening of the ethylcellulose. Also, residual solvent levels in coated drug resinate beads tend to be high due to both affinity of the polymer for the solvent and entrapment of solvent in agglomerated bead matrices. Therefore, the admixture solvent of ethanol and methylene chloride was used.

In the process of ethylcellulose film formation from mixtures of ethanol and methylene chloride, methylene chloride will evaporate first leaving ethylcellulose dissolved in ethanol. As the proportion of methylene chloride increases, this will ensure that at the stage at which the solvent system becomes rich in the poor solvent fraction, gel formation has occurred, no phase separation occurs and the formation of a close dense ethylcellulose film, as shown in Fig. 5. There will be a slow rate of drug release from the coated beads due to the homogeneous and dense film.

In the coating process, inlet air was kept at a temperature lower than the softening temperature of ethylcellulose film to avoid agglomeration of circulating drug resinate beads due to the softening of polymer

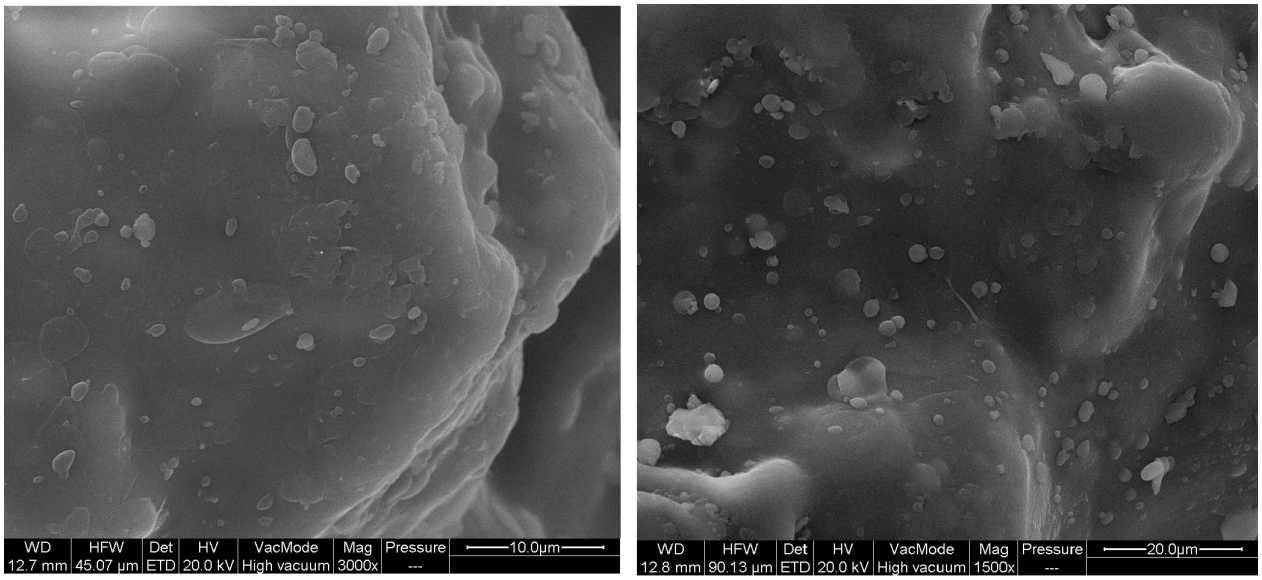

FIGURE 5 **Surface Morphology of Drug Resinate Beads Coated with Ethylcellulose.**

coat. Therefore, few significant agglomeration of circulating drug-resinate beads was observed during the operation, as shown in Fig. 6. PEG impregnation of drug resinate beads would be also effective for suppressing the agglomeration as expected (Ichikawa et al., 1997).

Residual Organic Solvents

Residual solvent concentrations at 5, 10, 15, and 20% coating levels with and without inprocess drying steps are summarized in Table 3. The results indicated the effect of coating level and drying steps on residual solvent concentration, and the residual solvent rose as coating level increased. This appeared to be related to poor drying efficiency of the more thick coating. A slight agglomeration observed likely tied up more solvent in the agglomerated bead matrices, and thus increasing the residual solvent. Additionally, residual solvent concentrations at the same coat levels with inprocess drying step were slightly decreased as anticipated, especially for the higher coating level.

Residual solvent in coating film, known as a potential toxic risk, could affect the glass transition temperature and permeability of the coating film, and the drug

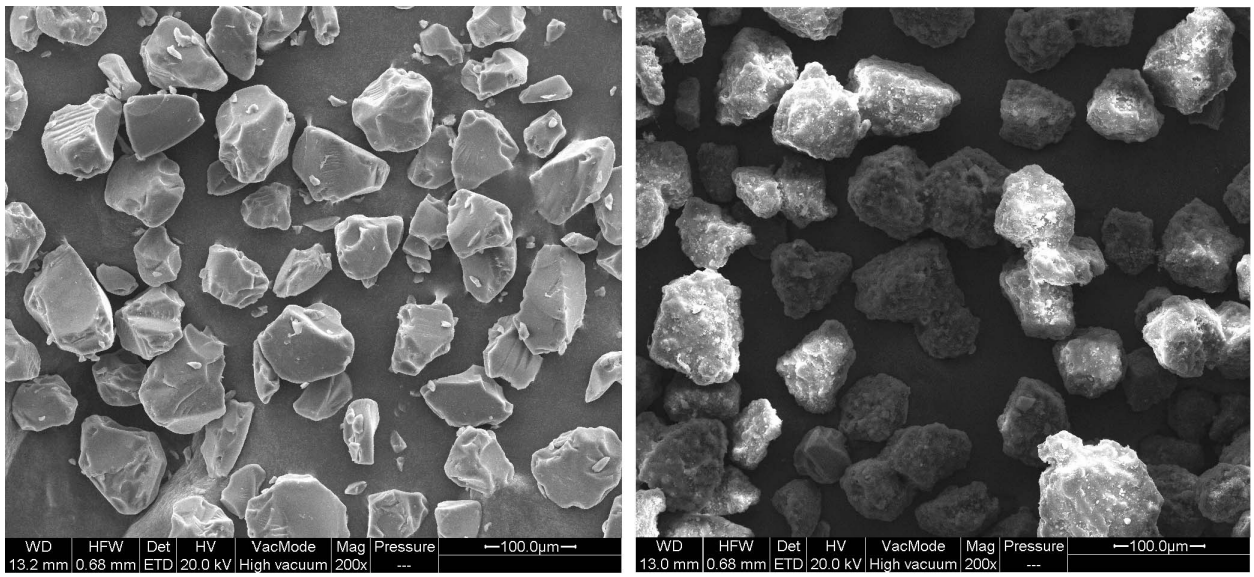

FIGURE 6 **Scanning Electron Micrographs of Drug Resinate Beads Before (Left) and After (Right) Coating.**

TABLE 3 **Residual Solvent Concentrations at Different Coating Levels without In-process Dry Step**

Coating levels (weight gain)	Residual solvent (ppm)	
	ethanol	methylene chloride
5%	459	78
10%	721	103
15%	925	114
20%	1036	126
20%*	793*	107*

*with in-process dry step.

release properties. A reasonable drying step should be included in the preparation to guarantee low residual solvents, and the content of residual solvents should be analyzed (Witschi & Doelker, 1997).

Preparation of CCSS

The CCSS was prepared by dispersing the coated PEG-impregnated drug resinate beads in an aqueous suspending vehicle containing 0.5% w/w xanthan gum and 0.5% w/w of hydroxypropyl methylcellulose of nominal viscosity 4000 cps, which allowed an adequate viscosity for oral administration, and also a good redispersability and high sedimentation time. The size of the coated PEG-impregnated drug resinates was under 200 μm, a size which allowed the coated drug resinate beads to be easily dispersed in a liquid suspension without leading to a gritty sensation during administration.

The CCSS was stored at 20°C and ambient humidity conditions for 6 months and at scheduled time intervals, samples were withdrawn and tested for their physical and release stability. The redispersability time showed practically no changes throughout the storage stability study. This indicated that the PEG-impregnated drug resinate beads coated by organic solvent based ethtylcellulose solution showed a hydrophobic surface that promoted the adsorption of the suspending polymer, thus avoiding their agglomeration and stabilizing the suspension. As shown in Fig. 7, the drug release profiles at the different storage time intervals of the CCSS were almost identical, and the results indicated the coating film of those resinate beads

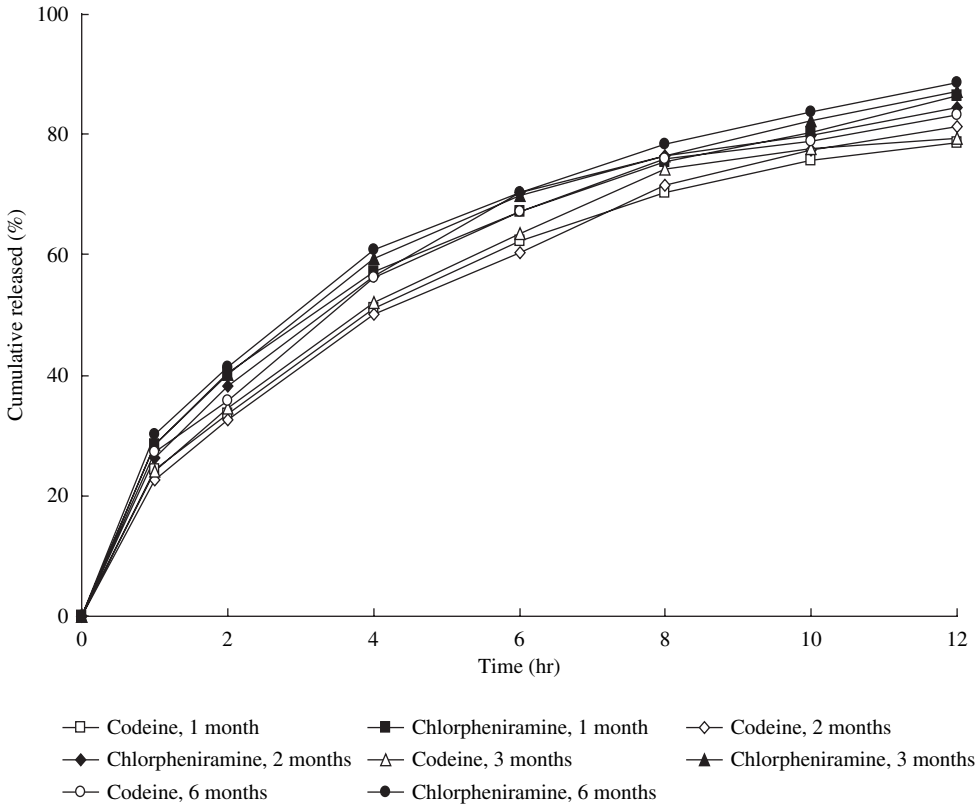

FIGURE 7 **Effect of Storage Time on the Drug Release from CCSS Prepared Using IERs as Carriers and Subsequent Fluidized Bed Coating.**

impregnated by PEG 4000 remained intact in aqueous suspending vehicle for 6 months of storage. PEG 4000 acts as an impregnating agent, and has an essential role in retaining the geometry of the beads when coating by the Wurster process techniques (Raghunathan et al., 1981; Chow & Raghunathan, 1990).In addition, the drug leaching to the suspending medium from the coated PEG-impregnated drug resinate beads in the suspension on storage for 6 months was practically negligible. In fact, the statistical analysis evidenced in this case that there was no influence of the storage time over the parameter of drug release behavior. Therefore, the CCSS formulated by coated PEG-impregnated drug resinate beads showed good release stability during the storage of 6 months.

In Vitro Release Studies

Drug release results of the drug resinate beads and PEG-impregnated drug resinate beads were shown in Fig. 1. It could be observed that codeine and chlorpheneramine released from their resinate beads quite rapidly with above 60% of the drug released within 1 hr, and those resinate beads impregnated by PEG 4000 alone did not retard the drug release. However, the combination of PEG 4000 impregnation followed by an ethylcellulose overcoat had a greater effect on control release of the drugs. Drug release profiles could be altered by varying the amount of ethylcellulose coating applied. As shown in Fig. 8, increasing the amount of ethylcellulose film on the PEG-impregnated drug resinate beads reduced the release rates of the highly water soluble drug codeine phosphate and chlorpheniramine maleate. The decrease in porosity resulting from the increase in polymer weight slows drug release. The weight gain of ethylcellulose film needed for a desired drug release profile depends on the characteristics of the drugs and IERs, amount and viscosity of ethylcellulose.

In Vivo Pharmacokinetics Studies

Using commercial immediate-release tablets as the reference preparation, the relative bioavailability and

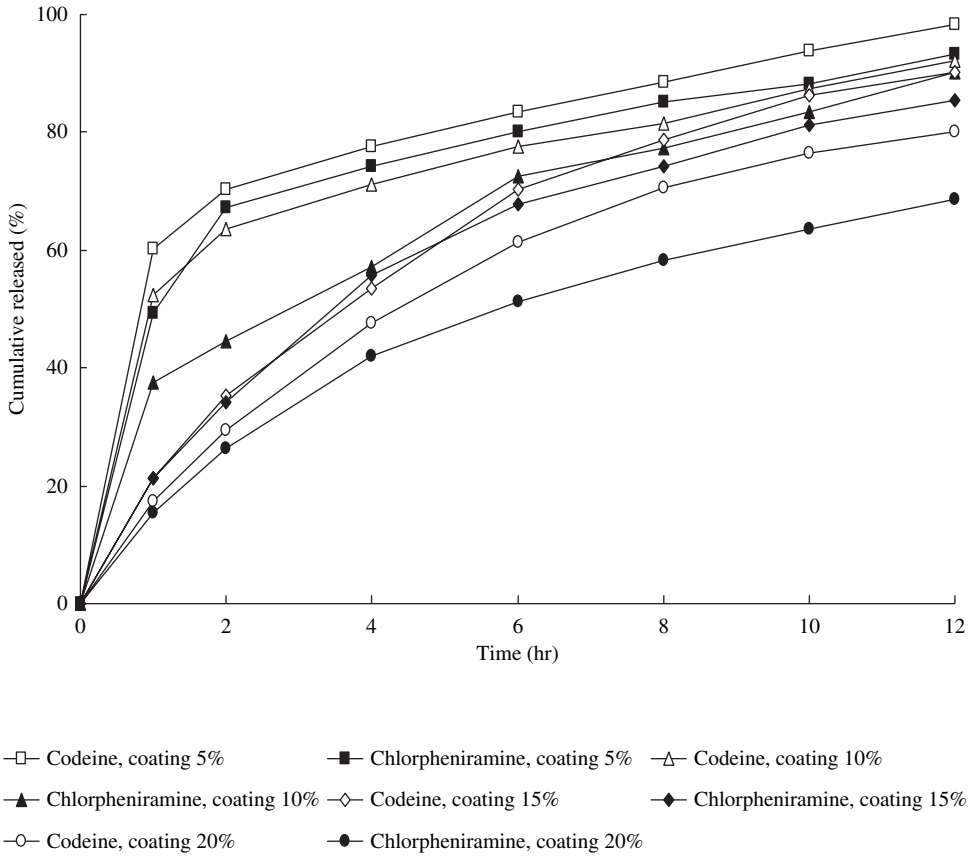

FIGURE 8 **Effect of Coating Film Weight Gained on the Drug Release from Coated PEG-Impregnated Drug Resinate Beads.**

pharmacokinetics evaluation of the CCSS were performed in beagle dogs. The plasma drug concentration data determined in beagle dogs after single oral administration of the CCSS are shown in Figs. 9 and 10. The following pharmacokinetics parameters were evaluated for both the test and reference products: the maximum concentration of the drug in plasma (C_{max}), the time to C_{max} (T_{max}) and the area under the curve from 0–24 hr ($AUC_{0\rightarrow24}$). The maximum plasma concentrations and the corresponding times were calculated by means of the experimental data. The areas under the plasma concentration-time curves from 0–24 hr ($AUC_{0\rightarrow24}$) were calculated by the linear trapezoidal rule. The terminal elimination half-lives ($t_{1/2}$) of the active pharmaceutical ingredients were determined via log-linear regression. Statistical data analysis was performed using the *t*-test with $p < 0.05$ as the minimal level of significance. The main pharmacokinetic parameters are shown in Tables 4 and 5. As could be seen from the Figs. 9 and 10, initial pronounced peaks obtained from the reference products (immediate-release dosage form) were eliminated in the test products (sustained-release dosage form), and the test products gave a flattened drug concentration–time profiles. On the other hand, the reference products showed distinct peak and then followed trough concentrations with the C_{max} of 18.36 μg/L at T_{max} of 0.59 hr and C_{max} of 5.88 μg/L at T_{max} of 0.91 hr for codeine and chlorpheniramine, respectively. However, in the case of the test products there were no distinct C_{max} peaks as the concentration–time curves were flat and concentrations attained in initial hours were maintained

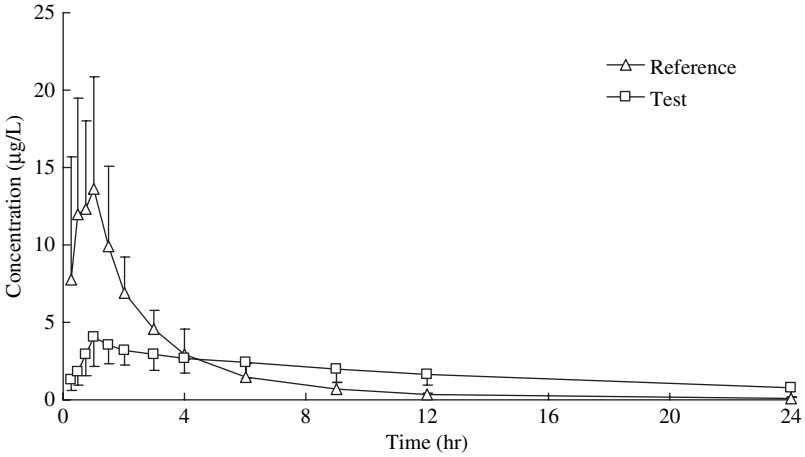

FIGURE 9 **The Mean Codeine Plasma Concentration (± SD) Time Profiles for CCSS and Immediate Release Reference Tablets After Peroral Single Dosing (Equivalent to 20 mg of Codeine) to Eight Healthy Adult Beagle Dogs.**

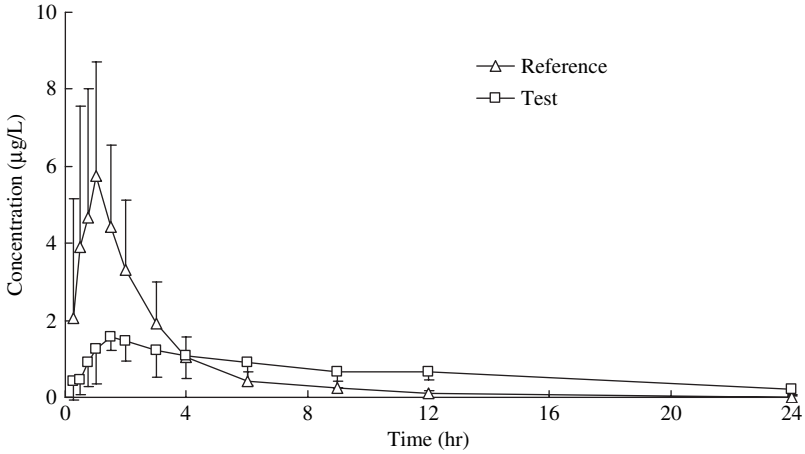

FIGURE 10 **The Mean Chlorpheniramine Plasma Concentration (± SD) Time Profiles for CCSS and Immediate-Release Reference Tablets After Peroral Single Dosing (Equivalent to 4 mg of Chlorpheniramine Maleate) to Eight Healthy Adult Beagle Dogs.**

TABLE 4 **The Pharmacokinetic Parameters of ccodeine Determined from Plasma Concentration-Time Profiles Obtained After Peroral Single Dosing (Equivalent to 20 mg of Codeine) of CCSS and Immediate-Release Reference Tablets to Eight Healthy Adult Beagle Dogs Following a Randomized Two-Way Cross-Over Design**

	T_{max} (h)		C_{max} (μg/L)		$t_{1/2}$ (hr)		$AUC_{0\rightarrow24}$ (μg/L·hr)		
Subject No.	Test	Reference	Test	Reference	Test	Reference	Test	Reference	F_r(%)
1	0.75	0.25	3.83	5.69	4.51	3.47	19.40	16.59	116.9
2	1.00	0.50	3.34	21.68	5.41	4.12	41.74	42.42	98.4
3	0.75	1.00	3.15	9.53	5.84	4.38	25.86	22.17	116.6
4	1.00	0.50	8.64	22.39	8.81	5.19	62.89	68.98	91.2
5	1.50	0.25	3.40	16.72	6.94	3.58	49.64	43.21	114.9
6	1.00	1.00	3.96	24.13	6.64	3.23	42.21	49.49	85.3
7	1.50	0.25	3.86	23.34	5.82	2.76	49.45	41.64	118.8
8	1.00	1.00	2.95	23.36	7.56	4.06	45.98	52.84	87.0
Mean	1.06	0.59	4.14	18.36	6.44	3.85	42.14	42.17	103.6
SD	0.29	0.35	1.85	7.09	1.35	0.75	13.82	16.66	14.6

TABLE 5 **The Pharmacokinetic Parameters of Chlorpheniramine Determined from Plasma Concentration-Time Profiles Obtained After Peroral Single Dosing (Equivalent to 4 mg of Chlorpheniramine Maleate) of CCSS and Immediate-Release Reference Tablets to Eight Healthy Adult Beagle Dogs Following a Randomized Two-Way Cross-Over Design**

	T_{max} (hr)		C_{max} (μg/L)		$t_{1/2}$ (hr)		$AUC_{0\rightarrow24}$ (μg/L·hr)		
Subject No.	Test	Reference	Test	Reference	Test	Reference	Test	Reference	F_r(%)
1	1.50	0.75	1.19	3.08	4.04	2.77	9.82	10.37	94.7
2	1.50	1.00	1.60	3.28	2.39	1.54	8.03	7.92	101.4
3	1.50	1.00	1.58	3.02	7.40	0.70	9.14	7.86	116.3
4	1.00	0.75	3.21	4.62	5.50	3.83	13.25	13.75	96.4
5	3.00	075	2.29	10.43	5.12	2.84	26.79	29.66	90.3
6	1.50	1.00	1.52	7.01	4.20	2.18	14.64	16.06	91.2
7	2.00	1.00	2.52	9.50	4.84	3.84	30.11	27.59	109.1
8	2.00	1.00	1.44	6.13	1.81	1.36	11.40	13.31	85.6
Mean	1.75	0.91	1.92	5.88	4.41	2.38	15.40	15.82	98.1
SD	0.60	0.13	1.92	2.92	1.77	1.15	8.38	8.42	10.3

till 12 hr. These results clearly demonstrated the sustained release of the active pharmaceutical ingredients and elimination of pronounced peak after administration of the CCSS.

As could be seen from the Figs. 9 and 10, the appearance of drug in the body would be quicker after administration of the reference products compared to the test products. Consequently, drug concentration would reach the minimum effective concentration more quickly and result in a reduced onset time. The peak concentration was also higher. Remember that the peak concentration of a drug is a function of the rate of absorption and the rate of elimination. Generally speaking, the rate of elimination would not change, the rate of absorption depends mainly on the rate of drug release from coated drug resinate beads, thus decreasing the rate of drug release will result in a lower peak concentration after administration, and a prolonged period of time during which the concentration of drug is above the minimum effective concentration, causing a longer duration of action for the drug. The results showed that the CCSS had the longer value of T_{max} and the lower value of C_{max}, which meant an obvious sustained-release effect, and the relative bioavailabilities (F_r) of codeine and chlorpheniramine were (103.6 ± 14.6)% and (98.1 ± 10.3)%, respectively, compared with the reference preparations. These findings indicated that a novel liquid sustained-release suspension made by using IERs as carriers and subsequent fluidized bed coating might provide a constant plasma level of the active pharmaceutical ingredients being highly beneficial for various therapeutic reasons.

Bioanalysis of Plasma Sample

The drug concentrations in plasma were measured by a LC-MS/MS method to determine the pharmacokinetic parameters of the CCSS. In our laboratory, sample analysis is always carried out in a GLP compliant manner and, therefore, the LC-MS/MS method needs to be validated according to currently accepted US Food and Drug Administration (FDA) bioanalytical method validation guidance (Guidance for Industry, 2001, 2003). The following parameters were considered as described below.

Linearity of the method was evaluated by preparing a series of standard solutions containing 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, and 100.0 ng/mL of codeine and corresponding 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 100.0 ng/mL of chlorpheniramine. These standard solutions were injected to HPLC in duplicate. Each peak area was tabulated in Microsoft excel spreadsheet program to make calibration curve for each drug. The square of the correlation coefficients and equations for the curves are shown in Table 6. From these results, it was acceptable to use a single point calibration in analysis of actual samples. The method had a lower limits of quantization of 0.1 ng/mL with a linear calibration range of 0.1–100.0 ng/mL for each drug. This method was also validated, and very good results, with respect to selectivity, precision and accuracy, were obtained for each of the analytes. The stability of the analytes in plasma under different temperature and time conditions, as well as the stability of the analytes in stock solution, was also evaluated, and the results showed this specific and precise method might be successfully applied to analyze plasma samples obtained after the administration of a single dose of the CCSS to eight healthy beagle dogs in a pharmacokinetic study.

TABLE 6 **Linearity Study Results of LC-MS/MS Analytical Method**

Analytes	Equation of calibration curve	Correlation coefficient squared (R^2)
Codeine	$Y = -0.0073715 + 0.0382263 \times X$	0.9977
Chlorpheniramine	$Y = -0.0140081 + 0.398749 \times X$	0.9981

CONCLUSIONS

A dual-drug sustained release suspension containing codeine and chlorpheniramine for oral administration, which provided a sustained release action for the drugs from coated drug resinate beads in the gastrointestinal tract, was prepared using IERs as carriers and subsequent fluidized bed coating.

The study revealed that IERs are useful alternative for liquid sustained release application with ethylcellulose coating, and PEG 4000 could be used to impregnate drug resinate beads to maintain their geometry and improve the coating process. Ethylcellulose film containing a suitable plasticizer exhibited excellent flexibility that could resist the expansion force resulting from the swelling of IERs.

The study also suggested combinations of ethylcellulose with di-n-butyl-phthalate as a coating formulation based on organic solvent mixture of ethanol and methylene chloride for the coating of drug-resinate beads could be suitable for a novel liquid sustained-release suspension.

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