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The influence of supercritical carbon dioxide in enhancing the dissolution rate of celecoxib from polyvinylpyrrolidone hot-melt-extruded tablets

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Objectives Hot-melt extrusion (HME) is a viable technology in producing solid molecular dispersions to enhance the oral bioavailability of poorly soluble drugs. HME produces too dense a structure, which might delay the drug-release rate especially from the extruded tablets. The aim of this work was to investigate the ability of supercritical carbon dioxide ($scCO_2$) to enhance the drug-release rate from hot-melt-extruded tablets.

Methods CX-PVPK25 (3:7 w/w ratio) was hot-melt extruded at 170°C and a screw speed of 100 rpm. The prepared melt extrudates were exposed to $scCO_2$ at 100 bar and 40°C. After 24 hours the chamber was evacuated rapidly of carbon dioxide and the samples were either cut into tablets or milled and sieved through mesh with a pore size of 250 μm. The samples of melt extrudates before and after $scCO_2$ exposure were characterized using differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), Fourier-transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). *In vitro* drug-release studies were done using simulated gastric fluid, adding 0.1% w/v Triton® X100 to provide sink conditions, and samples of equivalent weight of 50 mg celecoxib (CX) tablets and milled extrudates were tested.

Results The dissolution rate of CX from poly(vinyl pyrrolidone) (PVP) hot-melt-extruded tablets was enhanced significantly after $scCO_2$ exposure by around 2-fold after 2 hours compared with the non-exposed tablets. The milled tablets showed no significant difference in drug release either before or after $scCO_2$ exposure, while significant enhancement in drug-release rate was achieved compared with the corresponding physical mixture and CX powder. PXRD of melt-extruded samples showed a complete loss of CX crystallinity, with a single glass transition (T_g) appearing in the DSC. FTIR showed a significant shift in the carbonyl peak of PVP in the extruded samples. Samples exposed to $scCO_2$ showed significant porosity in SEM compared with the non-exposed samples, while there were no significant changes in the DSC, PXRD and FTIR results (Figure 1).

Conclusions $scCO_2$ enhanced significantly the release rate of solid molecular dispersions of CX from PVP hot-melt-extruded tablets by acting as an efficient pore-forming agent.

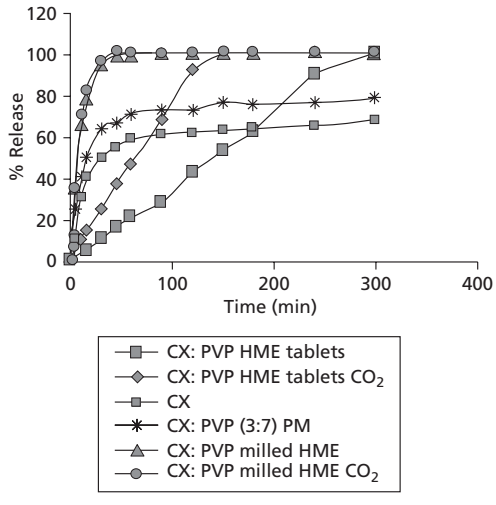

Figure 1 Drug release profiles of Celecoxib (CX) from hot melt extruded tablets before and after $scCO_2$ treatment compared with the physical mixture (PM) and the celecoxib (CX) powder. PM, physical mixture.

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Sodium calcium alginate as a matrix component of modified-release dosage forms: an evaluation of raw-material source change

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Objectives The biopolymer sodium calcium alginate is a modified-release matrix component for a range of monolithic oral dosage forms. Assessment of a proposed change to the raw-material source necessitated comprehensive physico-chemical and functional qualification to assure continued performance in the finished dosage form.

Table 1 Dissolution profile comparability analysis

Product	f_2 similarity factor	
	High shear	Low shear
Clarithromycin MR	80.1	91.8
Buflomedil CR	80.5	84.6

Methods For an alginate-based, modified-release dosage form, any significant change in alginate source, particularly to the seaweed feedstock, has potential to adversely impact dissolution performance of the finished dosage form. To qualify an alternative source, comprehensive comparative studies were performed including physico-chemical characterization and comparison. However, for dosage forms with multiple, complex mechanisms of release, prediction of final performance by means of simple physico-chemical comparison may be limited. Thus dissolution profile comparison (using f_2 similarity factor) from product manufactured on the pilot scale was utilized to complement physico-chemical comparability of key parameters including viscosity and calcium content, considered to directly influence functionality.

Results Particle-size analysis determined some small but potentially significant (Liew et al 2006) differences between sources. Physico-chemical comparison of sodium calcium alginate from an alternative source with that of the original source demonstrated a calcium content showing an apparent shift to a higher level. However, pilot-scale studies (Clarithromycin MR) using sieve fractions of an alternative source of sodium calcium alginate, screened above and below 63 μm, confirmed no statistically significant impact of particle-size distribution upon dissolution profile (f_2 similarity factor 65.2). Following pilot-scale manufacture using a range of production technologies, dissolution profiles for product containing an alternative source of sodium calcium alginate that reflected the extremes of the specification range for key physico-chemical parameters (viscosity, calcium content) were compared with original material. Despite minor differences in key physico-chemical parameters, resultant *in vitro* dissolution profiles show excellent similarity (Table 1).

Conclusions Despite differences in key physico-chemical parameters, *in vitro* dissolution comparability demonstrated no functional impact of a change in sodium calcium alginate source. Prediction of functional equivalence for differing sources of modified-release excipient also requires qualification by dissolution studies.

Liew, C.V. et al (2006) *Int. J. Pharm.* **309**: 25–37

Pharmacology

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Pharmacokinetics of clozapine in patients with schizophrenia

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Objectives The pharmacokinetic parameters of clozapine and its main metabolite, norclozapine, were evaluated in 37 selected chronic schizophrenic inpatients during long-term treatment. The dose–plasma-level relationship and inter- and intra-individual variability of plasma levels during maintenance treatment with clozapine were also investigated. The study had the approval of the university's ethics committee.

Methods Patients were all non-smokers and otherwise healthy males, aged 18–48 years, on clozapine monotherapy. All patients met DMS-IV criteria for diagnosis of schizophrenia and received clozapine every 12 hours (stable daily doses of 300–600 mg) for up to 2 years prior to the study. Serial blood samples were collected from each patient before the administration of the morning dose and 30 minutes and 1, 2, 3, 4, 5, 8 and 12 hours after. Plasma and red blood cell (RBC) drug concentrations were determined by high-performance liquid chromatography. All plasma drug concentrations were corrected for baseline (steady-state) values. The pharmacokinetic parameters were calculated from both non-compartmental and compartmental approaches with zero-order input rate using a kinetic model for simultaneous fit of clozapine and norclozapine concentrations.

Results Initial inter-patient variation observed in pharmacokinetic parameters of clozapine was reduced when the dose was normalized for body weight (BW) values. Plasma drug concentrations peaked, on average, at 2.1 hours. The mean volume of distribution and the total clearance, uncorrected for bioavailability and BW, were 7 L/kg and 55 L/hour, respectively. The terminal elimination half-lives averaged 7.7 hours for clozapine and 14 hours for norclozapine. The mean RBC/plasma concentration ratios were 24% for clozapine and 62% for norclozapine. From RBC concentration data, the mean elimination half-lives were 7.8 hours for clozapine and 16 hours for norclozapine. The average value for blood clearance of clozapine was 52 L/hour. Significant correlations were observed between dose and maximum plasma concentrations and between dose and area under the concentration/time curves ($P < 0.005$); these results suggested linear steady-state pharmacokinetics over the range of concentrations studied. The trough clozapine steady-state concentration averaged 360 ng/ml. The mean elimination rate constants from compartments 1 (k_{10}) and 2 (k_{20}, the elimination rate constant of norclozapine) were 0.085 and 0.16 hour^{-1}, respectively. The rate of formation of norclozapine, k_{12}, averaged 1.2 hour^{-1}. The mean fraction of the administered dose converted to norclozapine was estimated to be 65%. The sum of clozapine and norclozapine in urine represented on average 20% of the dose. Pharmacokinetics of clozapine were linear over the range of clozapine plasma concentrations of 145–1100 ng/ml.

Conclusions The pharmacokinetic model designed can be used to determine the population pharmacokinetic parameters of clozapine and norclozapine to optimize individual dosage regimens using a Bayesian methodology. The inter-patient variation found was partly attributed to the differences in patients' drug regimens and was not necessarily reflected in a change in psychopathology.

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The effects of pirenzepine on plasma gut-regulatory peptides and stress-related hormone levels in healthy humans

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Objectives Pirenzepine, an antagonist specific for muscarinic M_1 receptors, suppresses acid output and is used to treat gastritis and peptic ulcers clinically. Pirenzepine is also prescribed for the treatment of gastrointestinal symptoms caused by reduced gastrointestinal motility, upper abdominal pain, anorexia, heartburn, nausea and vomiting. In recent years some patients taking this medicine have not had organic disease such as reflex oesophagitis or gastric cancer but have had a condition classified as functional dyspepsia (FD). Most FD patients tend to have depressive and psychosomatic conditions and are exposed to continual affective stress. This continual stress causes abnormalities in the hypothalamo–pituitary–adrenal (HPA) axis and autonomic nervous function. However, the mechanism for gastric anti-secretory effects is not understood clearly. This study aimed to determine the effects of pirenzepine on plasma levels of motilin-, somatostatin-, calcitonin gene-related peptide-(CGRP-), substance P- and adrenocorticotropic hormone- (ACTH-)like immunoreactive substances (ISs) and cortisol under stress conditions in healthy subjects. Repetitive blood sampling places subjects under artificial stress, and venipuncture as a stressor is useful for the evaluation of the pharmacological effects of drugs. ACTH-IS is found in tissue other than the pituitary gland.

Methods Five healthy male volunteers participated in this study. Pirenzepine (75 mg) or placebo was orally administered to subjects with 100 mL water. Blood samples were taken before and at 20–240 minutes (seven times) after administration, and submitted to a highly sensitive enzyme immunoassay system for motilin-, somatostatin-, CGRP-, substance P- and ACTH-ISs. Plasma cortisol levels were measured using a fluorescence polarization immunoassay. Comparison of mean values was made by the Mann–Whitney U test and $P < 0.05$ was considered significant.

Results Peak plasma motilin-IS levels (110.0 ± 30.5 pg/mL) were achieved 180 minutes after administration of pirenzepine ($P < 0.05$ compared with the placebo), and returned to baseline levels (about 42.5 pg/mL). Plasma somatostatin-IS levels (33.1 ± 13.5 pg/mL) increased 90 minutes after administration of pirenzepine ($P < 0.01$ compared with the placebo, 11.2 ± 4.1 pg/mL). Plasma CGRP- and substance P-IS levels did not change significantly. A single administration of pirenzepine caused significant suppression of an increase in plasma ACTH-IS levels (about 8 pg/mL) at 60–90 minutes and tended to suppress increases in plasma cortisol levels (about 10 pg/mL) at 240 minutes, compared with the placebo response.

Conclusions Motilin is a powerful inducer of gastrointestinal motor activity in the fundus and the antral pouch of the stomach. In this study, because plasma motilin-IS levels significantly increased after a single administration of pirenzepine, the medicine might regulate gastrointestinal motility by accelerating gastric emptying. Somatostatin acts as an inhibitor of other hormone release. In this study, pirenzepine raised plasma somatostatin-IS levels. This increase might contribute to changing gastrointestinal functions through the effects of somatostatin. Pirenzepine regulated plasma ACTH and cortisol levels under stress. In general, venipuncture for blood sampling is postulated to be a stress factor that can increase circulating ACTH and cortisol levels. Pirenzepine might influence corticotrophin-releasing hormone (CRH) or ACTH, which are upstream on the HPA axis compared with cortisol. In this study, repetitive blood sampling resulted in an increase in ACTH (60 and 90 minutes) and cortisol (240 minutes) levels. Changes in plasma ACTH levels were more rapid than those in cortisol, and CRH levels might change before the changes in ACTH and cortisol. On the effects of pirenzepine for the HPA axis, this medicine stimulates the secretion and synthesis of pituitary ACTH, and the effects are mediated by hypothalamic CRH. These modulatory effects might be beneficial in stress-related diseases. We hypothesized that pharmacological effects of pirenzepine might be closely related to changes in motilin-, somatostatin- and ACTH-IS, and cortisol levels, which are related to regulation of gastrointestinal function and autonomic nervous function.

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Mast-cell-stabilizing activity of various extracts of *Ficus bengalensis* (Linn bark)

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Objectives Medicinal plants used for the treatment of asthma should have anti-inflammatory, immunomodulatory, anti-oxidant and anti-histaminic activities. *Ficus bengalensis* is used in treating Kapha, inflammation and ulcers, and as an immunomodulatory and anti-oxidant agent. The objective of the present study was to evaluate the anti-asthmatic activity of *F. bengalensis* bark using mast-cell degranulation and carageenan-induced inflammatory activity.

Methods Male Swiss albino mice weighing 25–28 g were used. A 3 day drug treatment schedule was followed. Group I received vehicle (normal saline; 5 mL/kg, intraperitoneally (i.p)). Group II were treated with disodium cromoglycate (200 μg/kg, i.p.). Groups III–VII were treated with aqueous, methanol, ethyl acetate, chloroform and petroleum ether extracts, respectively, in doses of 100 mg/kg, i.p. On day 4, each animal was injected with 4 mL/kg 0.9% saline solution into the peritoneal cavity by gentle massage; peritoneal fluid was collected after 5 minutes and transferred to a siliconized test tube containing 7–10 mL buffer medium (pH 7.2–7.4). This solution was then centrifuged at 400–500 rpm. Pellets of mast cell were washed with the same buffer medium twice by centrifugation, discarding the supernatant. These cells were challenged with clonidine (50 μg), incubated at 37°C in a water bath for 10 minutes, and then stained with 1% toludine blue and observed under a microscope (45×). A total of 100 cells was counted from different visual areas. Percentage protection against degranulation was calculated. The animal ethical committee of our institute approved all the protocols used in the study.

Results Clonidine degranulates mast cells via α-2 receptor agonist activity. Mice pre-treated with disodium cromoglycate (76.34 ± 0.8819%), aqueous (74.0 ± 1.155%), ethyl acetate (64.0 ± 1.528%) and methanol (65.34 ± 1.856%) extracts showed prevention of mast-cell degranulation to a significantly greater extent ($P < 0.01$) than the control group (19.0 ± 0.5774%), while chloroform and petroleum ether extracts failed to show statistical significance (see Table 1).

Table 1 Effect of various extracts of *F. bengalensis* bark on clonidine-induced mast-cell degranulation in mice. n = 5 animals in each group, *$P < 0.01$ compared with vehicle-treated group (one-way analysis of variance followed by Dunnett's test)

Group	Treatment (dose; all i.p.)	Protection of degranulated mast cells (%; mean ± SEM)
I	Vehicle (5 mL/kg)	19.0 ± 0.6
II	Disodium cromoglycate (200 μg/kg)	76.34 ± 0.88*
III	Aqueous extract (100 mg/kg)	74.0 ± 1.2*
IV	Methanol extract (100 mg/kg)	65.34 ± 1.86*
V	Ethyl acetate extract (100 mg/kg)	64.0 ± 1.5*
VI	Chloroform extract (100 mg/kg)	23.67 ± 0.88
VII	Petroleum ether extract (100 mg/kg)	24.0 ± 2.2

Conclusions The present study showed that only polar constituents prevent mast-cell degranulation whereas non-polar constituents were unable to prevent mast-cell degranulation.

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Investigations on wound-healing activity of leaves of *Ocimum basilicum* L.

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Objectives Indian basil or *Ocimum basilicum* L. is a holy plant and widely used to cure various ailments including skin diseases. In the present investigations, scientific validation of the wound-healing properties of leaves of *O. basilicum* was carried out along with investigation into the quality parameters of the extracts used for the study.

Methods Fresh leaves of *Ocimum basilicum* L. were collected, dried, powdered and extracted with three different solvents (water, alcohol and hydro-alcohol (25%)) by maceration for 72 hours with occasional shaking. All the extracts were concentrated under reduced pressure and at lower temperature using a rotary evaporator; after this they were dried. High-performance thin-layer chromatography fingerprinting of the dried extracts was developed. Swiss albino mice of 25–30 g in weight were used for the experiment and approved by the ethical committee of our institute. Animals were anaesthetized under ketamine anaesthesia (intraperitoneal 10 mg/kg) and an area of approximately 4 cm^2 on the dorsal surface was clipped free of hair and swabbed with 70% ethanol. The cleared dorsal surface of the skin was marked with a sterile circular (10 mm diameter) stainless steel stencil. A full-thickness wound was created by excising the skin flap in an aseptic environment using sterile scissors and forceps. The mice were divided into five groups (n = 5). Group I was kept as a control group, group II was kept as a positive control and treated with marketed formulation (0.5% w/w povidone iodine ointment) and groups III, IV and V were treated with the aqueous, hydro-alcoholic and alcoholic *O. basilicum* extracts topically on the wound daily up to the 16th day after wounding.

Results To evaluate the wound-healing potential of *O. basilicum*, hydroxyproline content and percentage wound contraction were estimated. Hydroxyproline content is an index of collagen and measures the synthesis of neocollagen, which is an indicator of improved condition of wound healing in animals. Hydroxyproline was estimated (Woessner 1961) from granulation tissues of animals on the 16th day after wound creation. The levels of hydroxyproline were found to be 6.40 ± 1.09, 7.10 ± 2.53, 10.11 ± 1.26, 14.95 ± 1.87 and 8.52 ± 1.28 mg/g of tissue in groups I–V, respectively. Hydroxyproline level was significantly higher ($P < 0.05$) in animals treated with hydro-alcoholic extract (group IV). Wound contraction indicates the rate of reduction of an unhealed area during the course of treatment; the greater the reduction the better the efficacy of the medication (Peacock 1984). Wound contraction was measured by tracing the wound margins at 4-day intervals on transparent graph paper with a millimetre scale to an accuracy of 0.05 mm. The wound-contraction data are shown in Table 1.

Conclusions In conclusion, the hydro-alcoholic extract of *O. basilicum* leaves showed significant wound-healing properties in this excision-wound model.

Table 1 Percentage of wound healing in mice from groups I–V

Group	Wound healing (%)			
	Day 4	Day 8	Day 12	Day 16
I (control)	43.68 ± 4.55	55.46 ± 2.01	72.52 ± 1.42	79.90 ± 0.82
II (positive control)	45.35 ± 4.53	60.76 ± 1.01	75.14 ± 1.46	82.70 ± 0.66
III (aqueous extract)	43.24 ± 3.42	69.36 ± 2.44	78.10 ± 0.70	86.52 ± 0.71
IV (hydro-alcoholic extract)	43.38 ± 1.86	74.58 ± 4.25	95.55 ± 0.85	98.42 ± 0.15
V (alcoholic extract)	40.72 ± 1.90	65.42 ± 4.19	76.78 ± 1.48	83.36 ± 1.04

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SESSION 2
Analytical Chemistry

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High-performance liquid chromatography method for simultaneous determination of gallic acid and ellagic acid in herbal extract and formulations

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Objectives This study was performed for the development and validation of reverse-phase high-performance liquid chromatography (RP-HPLC) methods for standardization of two widely used herbs, the whole plant of *Phyllanthus amarus* L. and seed extract of *Syzygium cumini* L. Two marker compounds, gallic acid and ellagic acid, were quantified from the methanol extracts as well as from commercial formulations using RP-HPLC. This paper presents an isocratic elution system for the separation of gallic acid and ellagic acid. Method validation was performed as recommended in the International Conference on Harmonisation (ICH) guidelines.

Methods Reference standards of gallic acid and ellagic acid (purity 98%, w/w) were from Natural Remedies (India) and all chemicals used were of analytical grade. Methanol extracts were prepared from the whole plant of *P. amarus* and seed of *S. cumini* and their formulations from three different places. A Thermo ODS Hypersil C_{18} (250 mm × 4.6 mm, 5 μm) column was used. Separation was achieved using a mobile phase consisting of 5 mM potassium dihydrogen phosphate (pH 3.2)/acetonitrile (41:9) at a flow rate of 1.0 mL/minute. The eluent was monitored using UV detection at a wavelength of 280 nm. Calibration curves were prepared using standard solution in a range of 0.8–8 μg/mL for gallic acid and 2–20 μg/mL for ellagic acid. The validation parameters addressed were specificity, precision, accuracy, linearity, limits of detection and quantification and the stability of gallic acid and ellagic acid in the mobile phase.

Results HPLC method development and optimization were done after tracking several elution systems. The study revealed that the gallic acid and ellagic acid were well resolved from *P. amarus* and *S. cumini* extracts as well as their formulations, with retention times of 3.8 and 10.3 minutes respectively. Good linearity was achieved in the investigated ranges for both analytes. Correlation coefficients were $R^2 = 0.998$ for gallic acid and $R^2 = 0.999$ for ellagic acid. The values for limit of detection (LOD) were 0.2 and 1 and limit of quantification (LOQ) values were 0.8 and 1.6 respectively. The relative SD (RSD) values for injection repeatability, analysis repeatability and for the intra-assay and inter-assay precision were lower than 2.0% of the peak area. The percentage recovery values at three different levels of gallic acid were 101.33, 99.04 and 101.14% and for ellagic acid were 97.20, 101.38 and 99.00%. The gallic acid and ellagic acid contents found were 0.41 ± 0.03 and 0.13 ± 0.02% respectively in *P. amarus* and 1.15 ± 0.02 and 0.36 ± 0.01% in *S. cumini* respectively.

Conclusions The developed HPLC method is precise, specific and accurate for determination of both the marker compounds. In previously reported work the time required for each run was high. But the method that we have developed proves that repeatable and selective analysis of these two active components in herbal drugs as well as in pharmaceutical formulations requires less time. It can also be used in routine quality control of herbal raw materials as well as formulations containing either or both of these compounds.

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The effect of complex coordination on gallium determination using ion chromatography

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Objectives Interest in the therapeutic use of gallium is increasing due to the discovery of its effectiveness as an anti-tumour and anti-microbial agent (Bernstein 1998). However, gallium strongly coordinates with many organic and inorganic ligands and these complexes can display very different physico-chemical properties (Houghton 1979). The gallium species present may also influence the accuracy of the assay used to quantify it. To enable the design of dosage form gallium must be accurately quantified irrespective of the coordination species present. The aim of this study was to develop a simple, rapid method for determining gallium content in a range of coordination complexes in a simple vehicle and a biological matrix.