Journal of Chromatographic Science, Vol. 48, October 2010

# LC Determination of Entacapone in Tablets: In Vitro Dissolution Studies

**Clésio S. Paim*, Magda T. Martins, Marcelo D. Malesuik,** and **Martin Steppe**

Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre-RS, Brazil

## Abstract

**The aim of the study was to develop and validate a dissolution procedure for entacapone-coated tablets. Several conditions such as medium composition, pH, surfactant concentration, and rotation speed were evaluated. The best dissolution conditions were achieved using apparatus 2, 900 mL of medium containing acetate buffer pH 5.3 at a rotation speed of 50 rpm, and a reversed-phase liquid chromatographic method for the quantification of the drug from the dissolution test, as well as to evaluate the dissolution profiles for tablets. The procedure was validated by specificity, linearity, accuracy, repeatability, intermediate precision, and robustness. The chromatographic method employed an Agilent Eclipse XDB RP-18 (150 × 4.6 mm i.d., particle size 5 µm) with a mobile phase consisting of water pH 3.0 and acetonitrile (65:35, v/v) at a flow rate of 2.0 mL/min. The dissolution procedure developed and validated was adequate for its purpose and could be applied for quality control for entacapone-coated tablets because there is no official monograph.**

## Introduction

Entacapone, (*E*)-2-cyano-*N*,*N*-diethyl-3-(3,4-dihidroxy-5-nitrophenyl)acrylamide (Figure 1) is an adjunct to levodopa therapy in the treatment of Parkinson's disease (PD). The drug is a potent, specific, and orally acting peripheral catechol-*O*-methyltransferase (COMT) inhibitor, an enzyme involved in the metabolism of dopamine and levodopa (1).

Entacapone is administered concomitantly with levodopa and carbidopa and prolongs the clinical effect of each levodopa dose by 30 to 40 min. When levodopa is administered in several frequent daily doses, addition of entacapone reduces the daily fluctuations of plasma levodopa by 30 to 40%. Based on studies with home diaries, entacapone increases the daily on-time by an average of 1–2 h and reduces the daily off-time correspondingly in patients with PD with motor fluctuations (2).

The dissolution tests for immediate-release solid oral dosage forms, such as tablets, are used to assess lot-to-lot quality of a drug product, guide development of new formulations, and ensure continuing product quality and performance after certain changes, such as changes in the formulation, and the manufacturing process (3). An appropriate dissolution profile approaches 100% recovery of the drug within 45 to 60 min. If the dissolution proceeds too quickly, it may produce a profile that levels off to early to show discrimination between the formulations. If the dissolution proceeds too slowly, the dissolution apparatus rotational speed or dissolution medium may have to be changed to produce a discriminating dissolution profile (4). Besides, the knowledge of the physical-chemical properties of the drug, such as pka, solubility, relationship pH/surfactant, and stability in function of the pH, are very important (5).

Although there are many works describing the determination of entacapone in biological fluids (6–12) and pharmaceutical formulation (13,14), there are no studies describing a dissolution method. Due to this, a method was validated by specificity, linearity, accuracy, precision (repeatability and intermediate precision), and robustness.

Then, the aim of the present work is to develop and validate a dissolution method for quantification of entacapone in coated tablets using high-performance liquid chromatography (HPLC) with UV detection for analytical routine and quality control according to the official code (15).

## Materials and Methods

### Chemicals

Entacapone reference substance (98.0%) was characterized by differential scanning calorimetry (DSC), infrared spectroscopy (IR), nuclear magnetic resonance (NMR) spectroscopy, and non-aqueous titration of weak acids (14).

Comtan 200 mg of entacapone (Novartis) was purchased in the market. The excipients of the pharmaceutical formulation were mannitol, microcrystalline cellulose, croscarmellose sodium, polysorbate 80, hydrogenated vegetable oil, 85% glyc-

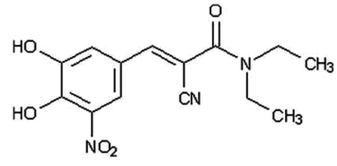


**Figure 1.** Chemical structure of entacapone.

*Author to whom correspondence should be addressed: email csoldatelli30@hotmail.com.

erol, sucrose, hydroxypropryl methylcellulose, magnesium stearate, titanium dioxide, and ferric oxides (yellow and red) as coloring agents. All of them were obtained from different local distributors. HPLC-grade acetonitrile and methanol were purchased from Tedia (Fairfield, OH). Orthophosphoric acid was purchased from Merck (Darmstadt, Germany). Sodium acetate, sodium hydroxide, monobasic potassium phosphate, and glacial acetic acid (reagent grade) were obtained from Nuclear (São Paulo, Brazil). High-purity water was prepared by using Millipore Milli-Q purification system (Billerica, MA). The buffers solutions were prepared in agreement with USP 31 (15). The dissolutions media were deuterated prior to use in the ultrasonic bath for 20 min.

**Instrumentation**

The dissolution test was performed in a Sotax AT7 multi-bath ($n$ = 7) dissolution test system (Basel, Switzerland) in accordance with USP general methods. Quantitative determination was performed in Agilent liquid chromatograph (Santa Clara, CA) equipped with a model Q 1311A quaternary pump, ALS-G1329A autosampler, TCC-G1316A column oven, and G1315B photodiode-array detector. ChemStation manager system software was used to control the equipment and to calculate data and responses from the HPLC system. A Shimadzu UV-Vis spectrophotometer UV-160A (Kyoto, Japan) at 385 nm and using 1.0-cm quartz cells and Spectra Manager software was used for all absorbance measurements. A Digimed potentiometer, model DM-20 (São Paulo, Brazil) was used to determine the pH of all solutions. The ultrasonic bath (Unique) used for deaeration was the model USC 5000 (São Paulo, Brazil). Sample filtration was carried out using a centrifuge Fanem model Excelsa 2 (São Paulo, Brazil). Framex (quantitative filter; 10 mm) and Millipore (0.45 µm; 13 mm; nylon membrane) were evaluated for sample filtration.

**Chromatographic conditions**

Chromatographic separation was performed on an Agilent Eclipse XDB RP-18 (150 × 4.6 mm i.d., 5 µm, Santa Clara, CA). The mobile phase comprised a mixture of water pH 3.0 adjusted with phosphoric acid 10% (v/v) and acetonitrile (65:35) at a flow rate of 2.0 mL/min with isocratic elution. The injection volume was 20 µL, and the run-time was 5 min. The temperature was set at 25°C in the column oven. Entacapone was determined by UV detection at 305 nm using photodiode-array. This wavelength was selected because it is a UV absorbance maximum and provides the sensitivity sufficient for quantitation of low drug concentration in dissolution samples. This quantification method was adapted from an HPLC method developed and validated in our laboratory to quantify entacapone in tablets (14).

**Dissolution test conditions**

The solubility of entacapone was determined in different dissolution médium, and the sink conditions were determined. Water, potassium biphthalate buffer (pH 3.5), acetate buffer (pH 4.5, 5.1, 5.3, and 5.5), phosphate buffer (pH 5.8, 6.0, 6.5, and 7.0), sodium lauryl sulfate (SLS) 0.25% (w/v) (pH 5.0), SLS 0.375% (w/v) (pH 5.0), and SLS 0.50% (w/v) (pH 5.0) were tested. Excess of entacapone reference substance (20 mg) was added in a tube containing 10 mL of medium test and maintained at 37 ± 0.5°C with shaking. An aliquot (5 mL) was removed from each tube after 1 and 2 h and then filtered. One milliliter of the filtered was transferred to a 50-mL volumetric flask, diluted with methanol, and analyzed by HPLC method, previously validated in our laboratory (14). According to Savolainen et al. (16) and Leppänen et al. (17), the solubility of the entacapone in acid medium is very low, and it was not evaluated.

Dissolution testing was performed in compliance with USP 31 (15) using apparatus 2 (paddles). A dissolution medium/agitation screen was performed for medium and paddle speed selection. Dissolution medium of acetate buffer (pH 5.3) was chosen based on the profiles obtained. A paddle speed of 50 rpm was used, and the medium, which was degassed under ultrasonic bath, was maintained at 37 ± 0.5°C. Samples were withdrawn at 5, 10, 15, 30, 60, and 120 min for early validation work. After dissolution optimization, aliquots were withdrawn at 3, 6, 9, 12, 15, 30, and 60 min. As entacapone tablets are immediate releasing, the earlier timepoints provided more discriminating ability. Manual sampling was performed using 10.0 mL aliquots, and these solutions were immediately filtered. Aliquots of 5.0 mL of these filtered solutions were transferred to 25-mL volumetric flasks and diluted with methanol, obtaining the final concentration of 44.44 µg/mL.

The reference standard solution, used in all dissolution tests, was prepared using 11.11 mg of entacapone that was transferred to a 50-mL volumetric flask with 5.0 mL of methanol. This solution was kept in an ultrasonic bath for 30 min, and the volume was completed with the dissolution medium (222.22 µg/mL). An aliquot (5.0 mL) of this reference standard solution was transferred to a 25-mL volumetric flask and diluted with methanol, obtaining the final concentration of 44.44 µg/mL. The reference standard and sample solutions were filtered in a 0.45-µm membrane before injection.

The filter evaluation is necessary to determine if it could be used in the dissolution test without adsorption of the drug and if it removes insoluble excipients that may otherwise cause high background or turbidity (15). Reference substance and sample solutions were prepared in a dissolution medium proposed with a final concentration of 44.44 µg/mL. The sample solutions were prepared using the equivalent to 11.11 mg of weight medium of the powered tablets, diluted in 50 mL of dissolution medium proposed, and maintained in ultrasonic bath for 30 min (Solution A). Aliquots of this solution were withdrawn and centrifuged. Another aliquot of the Solution A was withdrawn, filtered in quantitative filter, diluted in methanol, and filtered in a 0.45-µm membrane filter. The reference substance solutions were prepared in volumetric flasks, and the final solution was analyzed without filtration and filtered in a 0.45-µm membrane filter. All filtrates were analyzed by UV method. For a filter to be acceptable for use, the results of the filtered portions need to approach 98–102% the original concentrations of the unfiltered reference substance solution and the centrifuged sample solution (15).

The stability of reference substance and sample solutions at 222.22 µg/mL was evaluated at 37 ± 0.5°C for 2 h in the dissolution medium proposed. The stability in the diluted solutions (44.44 µg/mL) also was evaluated for 24 h at room temperature in the dissolution medium proposed and in methanol. After this time, the solutions were analyzed by HPLC

method, previously validated in our laboratory (14), to verify the peaks areas and the formation of degradation products.

## Validation of the Dissolution Method

In order to demonstrate the method was adequate for dissolution test purposes, it was validated through the analysis of specificity, linearity, precision, accuracy, and robustness, according to the USP 31 (15). The chromatographic parameters monitored were retention time, peak asymmetry, and theoretical plate number.

### Specificity

It was evaluated by preparing a placebo sample of commercial formulation of tablets in their usual concentration without the active ingredient. Samples of the placebo were transferred to separate vessels ($n$ = 3), filled with 900 mL of dissolution medium at 37 ± 0.5°C, and stirred for 1 h at 150 rpm using a paddle (USP apparatus 2). Aliquots of this solution were filtered and analyzed by HPLC–UV methods. Peak purity test, performed by photodiode array detector, was useful to show that the analyte chromatographic peak did not contain more than one substance.

### Linearity

A stock solution containing 200.0 µg/mL of entacapone reference substance was prepared in dissolution medium with 5% methanol to enhance drug solubility. The linearity of the method was evaluated in the 10–60 µg/mL range using dissolution medium. Each solution was prepared in triplicate. The linearity was verified by linear regression analysis, which was calculated by at least square regression method and analysis of variance (ANOVA).

### Precision

Precision of the method was determined by the repeatability and intermediate precision. Repeatability was determinate by replicate measurements of entacapone reference substance from linearity data. Intermediate precision was performed through the execution of the dissolution profile in different days by two analysts. Fresh samples and reference substance solutions were independently prepared on each day of analysis.

### Accuracy

Accuracy of the method was evaluated by the recovery test of known quantities of entacapone reference substance added to placebo solution at 80, 100, and 120% of the nominal assay of entacapone. Aliquots of 16, 20, and 24 mL of a 20 mg/mL entacapone reference substance solution dissolved in methanol was added to vessels containing dissolution medium for a final volume of 900 mL, pre-heated at 37 ± 0.5°C, and rotated for 1 h at 50 rpm. Aliquots were withdrawn, filtered, diluted in methanol, and analyzed by HPLC. Final concentrations were 35.56, 44.44, and 53.33 µg/mL, respectively.

### Robustness

The robustness of the method was verified through the analysis of the chromatograms obtained under small variations in the chromatographic conditions, such as pH of the mobile phase (2.8 and 3.2), percentage of acetonitrile in the mobile phase (33% and 37%), temperature of analysis (20°C and 30°C), flown (1.8 mL/min and 2.2 mL/min), stationary phase using a ACE octadecyl silane (150 × 4.6 mm, 5 µm, Aberdeen, Scotland), wavelength of detection (303 nm and 308 nm), and pH of the dissolution media (pH 5.1 and 5.5). For these studies, three determinations were accomplished.

## Results and Discussion

The entacapone biopharmaceutical classification is class IV (low solubility and low permeability) and because of that, the in vitro-in vivo correlations is limited or not expected (18). Therefore, the main objective of this study was to develop and validate a discriminatory dissolution method. The discriminatory power of the dissolution method depends on the ability of this method to detect changes in the drug product. Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium (15). The development of dissolution test was based on the physical-chemical properties of the entacapone, which is a weak acid and presents solubility dependent of the pH (18). The elevation of the pH increases the ionization and the solubility of the drug (19). Due to the pka of the drug, the solubility tests were accomplished one unit below of the pka (pH 3.5), in the pka (pH 4.5), and above that value (5.1 to 7.5). The use of SLS as dissolution medium was also evaluated due to the low solubility of the drug.

The solubility test showed that entacapone substance reference presented sink conditions only in phosphate buffer (pH 6.0), but the medium that fails to provide sink conditions may be acceptable if they showed to be more discriminating (15). Then, dissolution method for entacapone tablets was performed using the dissolution medium that showed total or near total solubility of entacapone (200 mg in 900 mL). The tested medium were as follows: acetate buffer (pH 5.1), acetate buffer (pH 5.3), acetate buffer (pH 5.5), phosphate buffer (pH 5.8), SLS 0.25% (w/v) (pH 5.0), SLS 0.375% (w/v) (pH 5.0), and SLS 0.5% (w/v) (pH 5.0).

The dissolution method conditions were selected based on a screening study with USP apparatus 2 at 50 rpm. First, the tablets were tested in 900 mL of acetate buffer (pH 5.1), acetate buffer (pH 5.3), acetate buffer (pH 5.5), and phosphate buffer (pH 5.8) (Figure 2A). The media contend different concentrations of

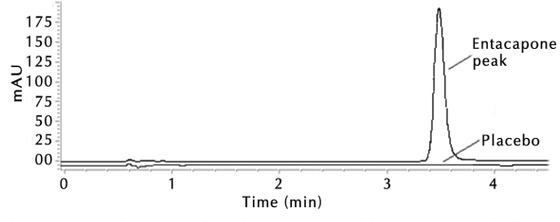


**Figure 2.** Dissolution profiles of entacapone tablets: (A) acetate buffer (pH 5.1), acetate buffer (pH 5.3), acetate buffer (pH 5.5), and phosphate buffer (pH 5.8) and (B) SLS 0.25%, SLS 0.375%, and SLS 0.5% using apparatus 2 rotating at 50 rpm.

SLS were also tested to verify the obtained profiles presented better discriminatory power (Figure 2B). In agreement with the results, the dissolution profiles that presented a larger capacity of discriminatory power were SLS 0.5% (w/v) (pH 5.0) and buffer acetate (pH 5.3) both using apparatus 2 rotating at 50 rpm. Table I demonstrates the likeness among the dissolution profiles obtained in these two medium. The choice of the acetate buffer (pH 5.3) was based on the effectiveness as a buffer of this medium compared to the solution of SLS, where a decrease of 0.3 units of pH was verified after the accomplishment of the dissolution method. Others decisive factors are due to the difficulty in working with solution of SLS due to formation of foam and the largest variability obtained (relative standard deviation, RSD) in the first points of the dissolution profile.

The total percentage of entacapone dissolved in acetate buffer (pH 5.3) was obtained between 30–60 min, which is appropriate according to Fortunado (4). The results demonstrated a fast liberation of entacapone in the first points of the dissolution profile (3–15 min), becoming slower with the development of the assay.

The use of the acetate buffer (pH 5.3) provided a dissolution profile with better discriminatory power in comparison to the other tested dissolution media, as well as the complete dissolution of the drug. Once there is only one entacapone brand in the Brazilian market, there is no need for comparison of dissolution profiles between products.

The evaluation of the filters demonstrated that the quantitative and 0.45-µm nylon membrane were within 98–102% of the initial values and could be used in the dissolution tests.

According to the literature (15), the acceptable range for solution stability is 98–102% of the initial value. The results demonstrated that the drug entacapone remained stable in acetate buffer (pH 5.3) for 2 h to 37 ± 0.5°C, but over 24 h, it degraded more than 2%. Due to this, the use of methanol was evaluated for accomplishment of the second dilution, and the obtained results demonstrated the stability of the drug in these conditions.

The specificity analysis revealed that UV method suffered interference by the excipients at 385 nm (maximum wavelength of entacapone in acetate buffer pH 5.3), but the HPLC method was specific because there was not another peak in the same retention time (3.5 min) of entacapone (Figure 3). The chromatographic peak purity tool, applied for entacapone peak, demonstrated that it was pure, confirming the absence of other substance coeluting in the same retention time. Thus, the HPLC method was useful in quantifying entacapone in pharmaceutical formulation from the dissolution method.

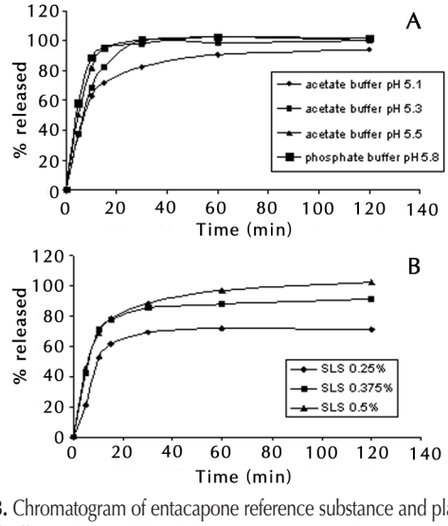


**Figure 3.** Chromatogram of entacapone reference substance and placebo in acetate buffer pH 5.3 at 37.0 ± 0.5°C using apparatus 2 rotating at 50 rpm.

To assess the linearity, the recommended range for the calibration curve is about 20% below the lowest and about 20% above the highest expected concentration of the dissolution test (15). The method demonstrated to be linear in the concentration range studied with a correlation coefficient of 0.99998. Linear regression was performed, and the equation obtained was $y = 33.98632x - 50.57224$. Data was validated by means of ANOVA, which showed significative linear regression and no-significative linearity deviation ($p < 0.05$).

The precision of the dissolution method was evaluated through repeatability and intermediate precision. The repeatability, evaluated through multiple injections accomplished for determination of the linearity of the method, demonstrated that the maximum RSD obtained was 1.58%. The intermediate precision results are shown in Table II. These results were considered acceptable.

Accuracy of the method was evaluated by the recovery test. For each level of the entacapone concentration, three determinations were performed. Recovery mean for entacapone were (mean% ± RSD) 99.83 ± 1.19, 100.36 ± 0.35, and 101.26 ± 0.58 for each level of the quantity, which indicated good accuracy of the method. Percentages of recoveries from 95.0 to 105.0% are recommended for the accuracy test (15).

**Table I. Comparative Results of Tablet's Dissolution Profiles***

| Time (min) | Acetate buffer pH 5.3 (% released) | RSD | SLS 0.5% (% released) | RSD |
|---|---|---|---|---|
| 3 | 22.40 | 33.45 | 17.87 | 40.27 |
| 6 | 60.90 | 12.95 | 58.28 | 17.23 |
| 9 | 77.19 | 7.57 | 77.33 | 9.87 |
| 12 | 84.01 | 5.77 | 84.78 | 6.24 |
| 15 | 90.06 | 4.15 | 89.32 | 3.72 |
| 30 | 95.01 | 2.73 | 98.90 | 2.57 |
| 60 | 99.44 | 2.59 | 102.44 | 0.97 |

* Using apparatus 2 rotating at 50 rpm ($n$ = 12).

**Table II. Intermediate Precision Results of the Dissolution Method in Acetate Buffer pH 5.3***

| Time (min) | Analyst A (n = 6) % dissolved | Analyst B (n = 6) % dissolved | Difference (%) between analysts |
|---|---|---|---|
| 3 | 20.32 | 24.49 | 4.17 |
| 6 | 59.60 | 62.19 | 2.59 |
| 9 | 75.15 | 79.24 | 4.09 |
| 12 | 81.84 | 86.20 | 4.36 |
| 15 | 89.23 | 90.90 | 1.67 |
| 30 | 94.76 | 95.27 | 0.51 |
| 60 | 100.13 | 98.75 | 1.38 |

* Using apparatus 2 rotating at 50 rpm.

The robustness of the method was demonstrated through the analysis of the chromatograms obtained under small variations in the chromatographic conditions. During these modifications, it was verified that small modifications in the pH of the dissolution medium can alter the liberation of the drug (Figure 2A). This factor should be controlled carefully due to the behavior of weak acid of the drug. The others results (Table III) confirmed the robustness of the test because during the modifications, the retention times, theoretical plates (N), and peak asymmetry (*T*) suffered very small variations. The statistic analysis demonstrated that the variability was low (RSD < 1.52) for the percentage of dissolution rate (% dissolved) in the robustness test. Then, it was possible to demonstrate that the developed method was robust with all the changes employed, except for the use of the pH of the dissolution medium.

The system suitability was verified through common parameters to entacapone peak. The approximate results were as follows: theoretical plates ($N = 4050$) and peak asymmetry ($T = 1.15$). The values for these parameters were satisfactory according to the literature (20,21). Typical acceptance criteria for the amount of active ingredient dissolved are in the range of 75–80% dissolved. This criterion including test times is usually established on the basis of an evaluation of the dissolution profile data (15). In this article, it was observed 90% of entacapone dissolved in 15 min.

## Conclusions

The dissolution method developed and validated for entacapone-coated tablets was considered satisfactory. The conditions of the dissolution method selected for Comtan coated tablets were 900 mL of medium containing acetate buffer (pH 5.3) and USP apparatus 2 at 50 rpm stirring speed. The dissolution method was successfully validated according to USP 31 (15), and the HPLC method showed to be specific, linear, precise, accurate, and robust, but the pH of the dissolution medium should be controlled carefully. Then, it can be recommended to be applied in routine quality control because there is, as yet, no official method.

**Table III. Robustness Test Results of the Dissolution Method in Acetate Buffer pH 5.3***

| Modification | Retention time (min) | Theoretical plates (N) | Asymmetry (T) | % dissolved† |
|---|---|---|---|---|
| Optimal experimental conditions | 3.605 | 4050 | 1.14 | 98.82 |
| Detector: λ 303 nm | 3.598 | 3661 | 1.15 | 97.88 |
| Detector: λ 308 nm | 3.609 | 3681 | 1.15 | 97.98 |
| *Mobile phase:* | | | | |
| water pH 3.0–ACN (63:37, v/v) | 3.011 | 3554 | 1.15 | 98.12 |
| water pH 3.0–ACN (67:33, v/v) | 4.390 | 3768 | 1.11 | 98.98 |
| Flow 1.8 mL/min | 4.834 | 3784 | 1.09 | 98.36 |
| Flow 2.2 mL/min | 3.295 | 3499 | 1.14 | 98.14 |
| Column ACE® | 4.073 | 3455 | 1.31 | 100.97 |
| *Mobile phase:* | | | | |
| water pH 3.2–ACN (65:35, v/v) | 3.568 | 4071 | 1.22 | 98.56 |
| water pH 2.8–ACN (65:35, v/v) | 3.627 | 4139 | 1.17 | 98.96 |
| Oven temp.: 20°C | 3.819 | 4190 | 1.12 | 98.54 |
| Oven temp.: 30°C | 3.450 | 3793 | 1.15 | 98.69 |

* Using apparatus 2 rotating at 50 rpm.
† Average of three tablets submitted to the dissolution test.

**Acknowledgments**

The authors wish to thank to LEPCQ, LCQFar, and Brazilian Pharmacopoeia by the financial support.

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Manuscript received November 19, 2008;
revision received March 10, 2009.