PAPER

COLORIMETRIC DETERMINATION OF PHOSPHORUS IN MILK BY FLOW INJECTION ANALYSIS USING A THERMAL/UV INDUCED DIGESTION

DETERMINAZIONE COLORIMETRICA DEL FOSFORO NEL LATTE MEDIANTE ANALISI AD INIEZIONE DI FLUSSO E DIGESTIONE TERMICA INDOTTA DA RADIAZIONE UV

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ABSTRACT

Two flow injection systems for the determination of total phosphorus in milk were developed: one for the determination of phosphorus using off-line digestion of milk and another with in-line digestion. The first procedure involves a classical Kjeldahl digestion of milk prior to introducing it into the flow system; the second uses acid, heat and UV-catalysed peroxodisulphate oxidation to convert in-line all forms of phosphorus compounds into orthophosphate, allowing a considerable amount of time to be saved. Concentrations of phosphorus in milk within

RIASSUNTO

Sono stati sviluppati dei sistemi ad iniezione di flusso (FIA) per la determinazione del fosforo totale nel latte. Nel primo la determinazione del fosforo avviene dopo che la digestione del latte è stata condotta a parte (off-line), mediante la classica reazione di Kjeldahl; nel secondo la digestione viene condotta direttamente nel sistema di flusso (in-line), trasformando tutti i composti di fosforo in ortofosfato mediante reazione a caldo con acido, catalizzata da radiazione UV, ed ossidazione con perossidisolfato. Quest'ultimo metodo consente un risparmio di tempo significa-

- Key words: Flow-injection, in-line UV/thermal digestion, milk, phosphorus determination, spectrophotometry -

the range of 706 to 984 mg P/L were found and FIA results were in good agreement with those provided by the reference method.

tivo. Le concentrazioni di fosforo trovate nel latte sono risultate comprese tra 706 a 984 mg di P/L. I risultati sono in buon accordo con quelli ottenuti applicando il metodo di riferimento.

INTRODUCTION

Milk is an exceptionally nutritious food primarily because it has a large number of components such as proteins, sugars, vitamins, fats, gases and minerals. However, this complex matrix poses a major problem to the analytical chemist.

The objective of this work was to develop an automatic method to determine the total phosphorus present in milk, a mineral essential to the human diet (PULLIAINEN and WALLIN, 1994). All food contains phosphorus in a minor or major quantity; ultra-pasteurised milk (UHT) contains from 65 to 100 mg of phosphorus per 100 g, in a Ca/P ratio of 1.4.

The spectrophotometric determination of phosphorus involves two main steps: (a) conversion of all forms of phosphorus into orthophosphates by hydrolysis and oxidation; (b) colorimetric measurement using the phosphomolybdenum blue reaction.

Flow injection systems have already been developed for the determination of phosphorus in beer, water and wastewater. They have been applied only to the colorimetric reaction (WOO and MAHER, 1995) using off-line sample pretreatment, or providing automation of the whole process (BENSON et al., 1996; FERNANDES et al., 2000; WILLIAMS et al., 1993). In none of these studies, however, was total phosphorus determined in milk samples. Therefore, it would be desirable to develop a system in which the design and conditions of the manifold would be able to handle the conversion of all forms of phosphorus into orthophosphate, in a complex matrix like milk.

In this work two flow injection analysis (FIA) systems are proposed for the determination of phosphorus in milk; the first one automates the colorimetric phosphomolybdenum blue reaction, being the digestion procedure performed off-line; the second one provides UV-photooxidation/thermal in-line digestion with oxidising and hydrolysing reagents to convert all forms of phosphorus compounds into orthophosphate and the subsequent colorimetric reaction, with the advantage of requiring minimum sample pre-treatment (dilution).

MATERIALS AND METHODS

Reagents and solutions

All chemicals were of analytical reagent grade. Deionised water with a specific conductance of less than 0.1 μS/cm was used throughout. The material used in the experiments was washed with water only and rinsed with 0.1 M H_2SO_4, as detergents highly interfere in these determinations as reported by PULLIAINEN and WALLIN (1994).

A 2 M H_2SO_4 solution was prepared by proper dilution of a concentrated sulphuric acid solution (d=1.98, 98%); a 6 g/L potassium peroxodisulphate solution was obtained by dissolving 3 g of the solid in 500 mL of water.

The phosphorus stock solution (1000 mg P/L) was prepared weekly by dissolving 4.3909 g (weighed in a Sartorius analytical balance) of potassium dihydrogen phosphate per liter of water. An intermediate solution (100 mg P/L) was

prepared daily by rigorous dilution of 10 mL of the stock solution to 100 mL. This solution was used to prepare the working standard solutions used in both manifolds: for the off-line digestion procedure, standards were prepared in the range of 8 to 25 mg P/L in a 0.75 M H_2SO_4 solution; for the in-line digestion method, standards of 6 to 25 mg P/L in water were used.

The colour reagent, a 9.4 g/L ammonium molybdate solution, was obtained by dissolving 5 g of ammonium heptamolybdate-tetrahydrate in 18 mL of concentrated sulphuric acid and diluted to 500 mL. This solution was 0.65 M in sulphuric acid and was prepared weekly.

The stannous chloride solution was obtained using 0.1 g of stannous chloride dihydrate and 1 g of hydrazinium sulphate (which improves the stability of the solution) dissolved in 14 mL of concentrated sulphuric acid; the volume was brought up to 500 mL with water. This solution, prepared every week, was 0.17 g/L in stannous chloride, 0.5 M in sulphuric acid and 2 g/L in hydrazinium sulphate.

For the reference procedure, the colour reagent was prepared by dissolving 6.25 g of sodium molybdate in a 5 M sulphuric acid solution. In a separate vessel, 0.15 g of hydrazinium sulphate was dissolved in 100 mL of water. Then, 25 mL of the molybdate solution was added to 10 mL of the hydrazinium solution and the volume was made up to 100 mL; this procedure was performed daily.

For the preparation of a 10 mg/L intermediate standard solution, 10 mL of the 100 mg/L stock solution prepared for the flow injection procedure were taken and diluted to 100 mL. This solution was used to prepare the working standard solutions from 0.2 to 2 mg P/L.

Instrumentation

In the flow injection systems the solutions were propelled by Gilson Minipuls 3 (Villiers-le-Bel, France) peristaltic pumps and Gilson PVC propelling tubes. The tubing was made of PTFE [Omnifit (Cambridge, UK), 0.8 mm i.d.] and Gilson end fitting connectors and Y-shaped confluences were used to link the different parts of the manifold.

The thermal digestion unit consisted of a 4 m long Omnifit PTFE helically coiled tubing (0.8 mm i.d.) submerged in a Julabo VC (Seelbach, Germany) thermostatic bath with temperature set at 90°C.

The UV digestion system consisted of a 4 m long Omnifit PTFE tubing (0.8 mm i.d.) helically coiled around a 15 W UV tube (Philips, Eindhoven, Holland). An aluminium foil covered this system to prevent the operator from being continuously exposed to this radiation.

An ATI Unicam (Cambridge, UK) 5625 UV/Vis spectrophotometer equipped with a Hellma (Müllheim/Baden, Germany) 178.713-QS flow cell (inner optical volume 18 μL) and connected to a Kipp and Zonen (Delft, Holland) BD chart recorder was used as detection system.

Samples were injected in the manifold using a Rheodyne (Cotati, CA, USA) type 5020 six-port rotary injection valve.

A Bandelin Sonorex (Berlin, Germany) RK 100 ultrasonic bath was used for degassing samples.

Reference method

The colorimetric procedure recommended by the Manuel Suisse des Denrées Alimentaires (MSDA, 1973) was used as the reference method. For preparing the sample, 2 mL of milk, 5 mL of concentrated sulphuric acid and 1 g of a catalyser (mixture of 500 g of anhydrous sodium sulphate, 8 g of copper sulphate and 8 g of selenium) were put into Kjeldahl tubes. This solution was mineralised at 400°C for about 3 h and allowed to cool. Then, 30 mL of water were added and the volume was brought to 100 mL.

From this solution, 2 mL were put in

50 mL volumetric flasks and 25 mL of water and 20 mL of the colour reagent were added. This solution was boiled in a water bath for about 10 min. After cooling, the volume was brought to 50 mL with water and the absorbance measured at 710 nm. The phosphorus content of the samples was then determined from a previously established calibration curve.

Sample preparation

The digestion procedure described above for the reference method was used for preparing the samples to inject in the manifold using off-line digestion.

For the in-line digestion procedure, 2 mL of milk were diluted to 100 mL in a volumetric flask and then degassed in an ultrasonic bath for ten minutes.

Flow injection procedures

The two flow injection systems are presented in Fig. 1. The flow injection procedure with off-line digestion (Fig. 1b) consisted in the mixture of the stannous chloride reducing reagent (R_4) and the molybdate ammonium solution (R_5) in the first confluence (d). The sample (30 µL of digested milk) was injected in the water carrier stream (R_3), merged at confluence (c) with the previously formed colour reagent and allowed to react in L_5 coil. Total phosphorus was then determined spectrophotometrically at 710 nm.

Fig. 1a shows the flow injection procedure with in-line digestion. The sample (S) was continuously mixed in confluence (a) with a 2 M H_2SO_4 stream (R_1) and then flowed through the thermostatic bath (L_1). This solution then merged with a potas-

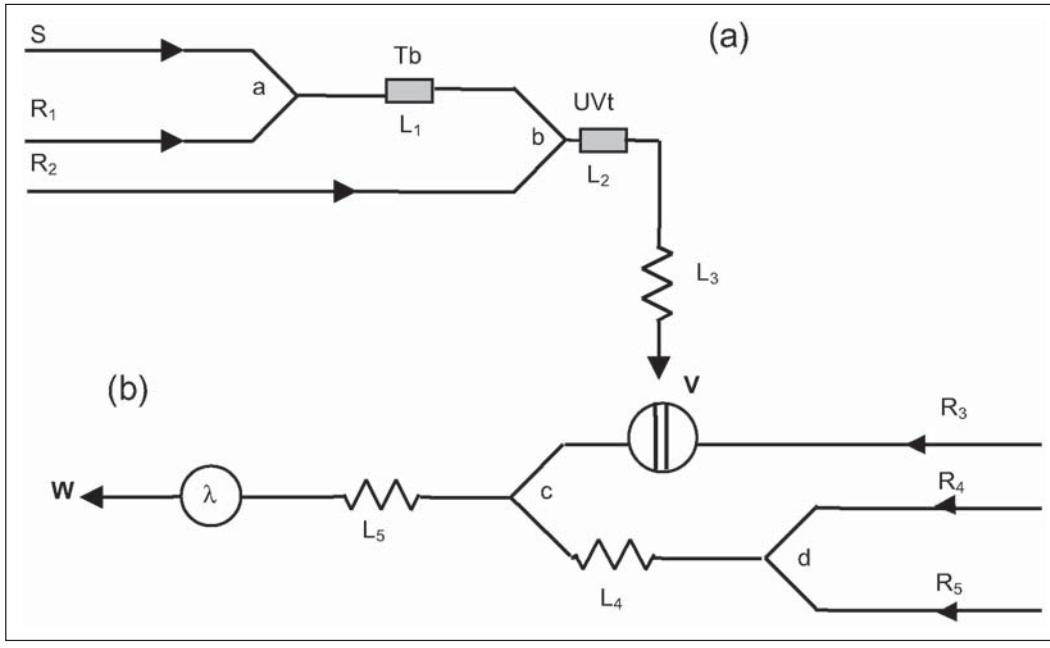

Fig. 1 - FIA manifold used for the determination of phosphorus in milk.
(a) - Digestion unit; (b) - Spectrophotometric determination unit; S - standards or milk samples; R_i - reagent solutions (R_1 = 2 M H_2SO_4; R_2 = 6 g/L potassium peroxodisulphate; R_3 = deionized water; R_4 = 0.17 g/L stannous chloride; R_5 = 9.4 g/L ammonium molybdate); L_i - tube lengths, in cm (L_1 = 400; L_2 = 400; L_3 = 100; L_4 = 200; L_5 = 200); V - injection volume (30 µL); W - waste; a, b, c and d: confluence points; UVt - ultra violet radiation tube; Tb - thermostatic bath (90°C); λ - UV/Vis spectrophotometer.

sium peroxodisulphate solution (R_2) and flowed through the coil (L_2) wrapped around the UV tube. After this point, an aliquot (30 µL) of the digested sample was injected in the manifold described above for the off-line procedure.

RESULTS AND DISCUSSION

Manifold optimisation tests were made by varying each parameter while setting the remainder.

Study of the flow injection system using off-line digestion

The system parameters were studied in order to achieve a compromise between sensitivity of the analytical measurements, repeatability and sampling rate. Since the phosphomolybdenum blue reaction has been widely studied in flow injection systems (LIMA and RANGEL, 1990), the concentrations of the colour and reducing reagents were based on literature values.

The flow rates in channels R_3, R_4 and R_5 were made equal and set at 1.1 mL/min. The length of the coil L_5 was set at 200 cm since it provided the best sensitivity (FERNANDES et al., 2000).

The loop of the injection volume was tested from 25 to 45 µL. It was set at 30 µL as higher injection volumes decreased sensitivity due to the increasing amount of H^+ of the injected plug. Smaller volumes also produced a decrease in sensitivity.

Since the acidity of the medium strongly influences the development of the colorimetric reaction (LIMA and RANGEL, 1990), working standard solutions were prepared with different levels of acidity (from 0.4 to 1.1 M in H_2SO_4) and injected in the manifold. In fact, increasing the acidity of the injected standards strongly decreased the sensitivity of the system (Fig. 2). Considering that the digested samples injected into the system might have different acidities, this parameter was assessed by titration with NaOH. It was found that all digested samples had an acidity of around 0.75 M, expressed in H_2SO_4. This way, the acidity of the standard solutions was matched to that of the injected samples, by preparing flow injection standards in a 0.75 M H_2SO_4 solution.

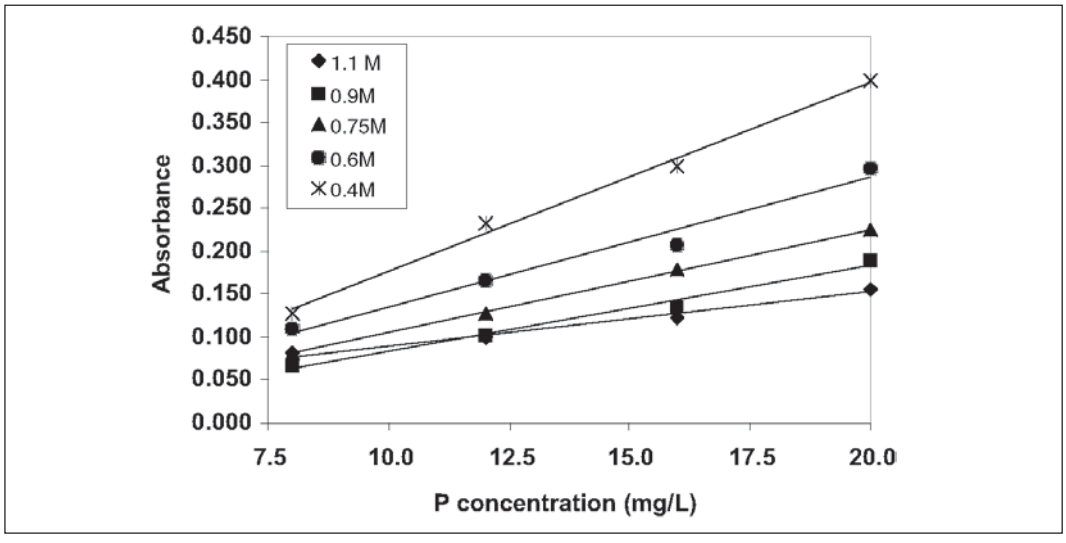

Fig. 2 - Influence of the acidity (expressed in H_2SO_4 molarity) of the injected solutions on the colorimetric determination of phosphorus.

Under the optimised conditions, a sampling rate of 30 samples per hour was achieved with this FIA system. The limit of detection was calculated according to IUPAC (1976) recommendations, corresponding to 3 times the standard deviation of ten consecutive injections of the blank solution (0.75 M H_2SO_4) and was found to be 2 mg/L.

Flow injection system using in-line digestion

For the development of this manifold, the spectrophotometric determination was accomplished by using the system previously described for off-line digestion (Fig. 1b). This way, the only parameters studied were those related to the in-line digestion procedure, seeking to obtain results comparable to the ones achieved with the reference digestion method.

The length of tube L_1 was tested up to 4 m. This length was chosen as it allowed a higher conversion percentage of the phosphorus compounds, because of the increased contact time of the milk with the acid and heat. Tube L_2 was fixed at 4 m, as reported by FERNANDES et al. (2000).

Since one of the factors affecting the rate of hydrolytic degradation of ring and chain phosphates in solution is pH (Williams et al., 1993), the H_2SO_4 concentration was also studied. As reported by FERNANDES et al. (2000), a 2 M H_2SO_4 concentration was enough to convert all forms of phosphorus into orthophosphates. Higher concentrations caused double peaks due to refractive index gradients without increasing the conversion percentage.

Concerning the potassium peroxidisulphate solution, it was tested in the range of 6 to 15 g/L. More than 6 g/L led to a decrease in the sensitivity of the spectrophotometric determination and did not improve the conversion of phosphorus compounds. Under the optimised conditions, the detection limit of this methodology was 4 mg P/L, calculated according to the IUPAC (1976) recommendations.

Analysis of milk samples

Determinations of total phosphorus in milk samples were carried out using both FIA procedures (C_f) and the reference method (C_r). Each standard or milk digestate was injected three times (Fig. 3). Several commercially available types were analysed, including whole milk, reduced fat milk, skimmed milk and different special brand milks. Results are shown in Tables 1 and 2 for off-line and in-line digestion FIA manifolds, respectively.

In order to assess the quality of the FIA results, a relation of type $C_f=C_0+SC_r$ was established, where: C_0=-40 (±180), S=1.04 (±0.20) and r=0.974 for the off-line digestion procedure, and C_0=9 (±112), S=0.973 (±0.154) and r=0.969 for the in-line digestion procedure. The confidence limits of the intercept and slope obtained with a 95% level of significance are in parentheses (MILLER and MILLER, 1993) for 8 and 12 degrees of freedom, respectively. These values show that there was no evidence of systematic differences between the results obtained using the two methodologies. The maxi-

Table 1 - Results for the determination of phosphorus in milk by the off-line digestion FIA system and the reference method.

Samples	Ref. Method (mg/L)	FIA/off-line digestion (mg/L)	Rel. deviation (%)
1	888	864	-2.7
2	983	978	-0.51
3	985	984	-0.10
4	871	845	-3.0
5	858	859	+0.12
6	905	926	+2.3
7	824	813	-1.3
8	914	923	+0.98
9	969	955	-1.4
10	847	841	-0.71

mum relative deviation, calculated as $[(C_f-C_r)/C_r] \times 100$, was 6.2%.

To assess the repeatability of the FIA procedures, the relative standard deviations were calculated from ten consecutive determinations of milk samples. The results were: 0.30, 0.45 and 0.54% for the off-line digestion procedure, with concentrations of 816, 825 and 834 mg P/L respectively, and 0.42 and 0.66% for the in-line digestion procedure, with concentrations of 753 and 668 mg P/L, respectively.

CONCLUSIONS

It can be concluded that both FIA systems are advantageous alternatives to the reference method, featuring all the advantages of automated methods. For the first time a flow injection in-line UV/thermal digestion procedure was successfully applied to the digestion of a complex matrix like milk, yielding results comparable to those of conventional digestion methods and having good precision. This system presents the advan-

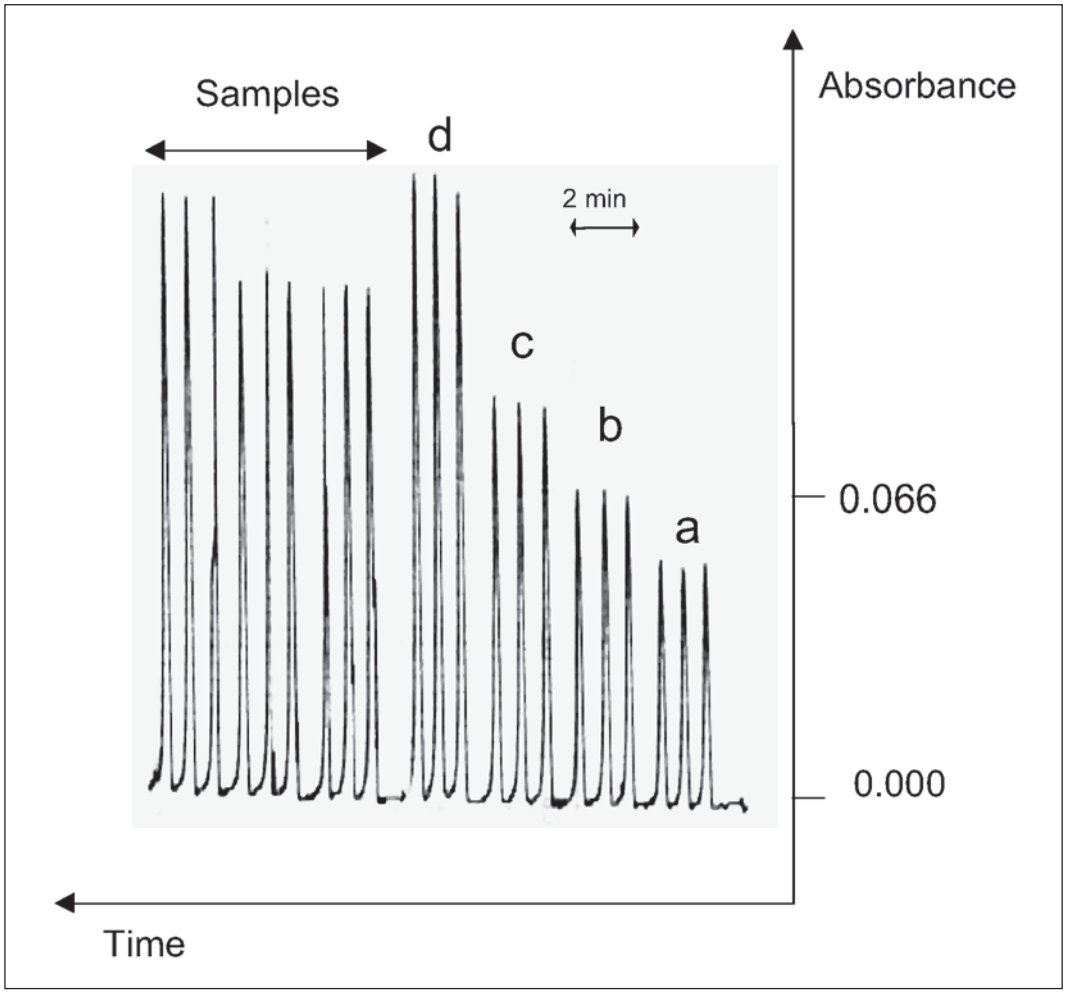

Fig. 3 - FIA recording obtained for determination of total phosphorus in milk with the in-line digestion manifold, corresponding to injections of samples and standards: 6 (a), 8 (b), 10 (c) and 15 (d) mg/L.

Table 2 - Results for the determination of phosphorus in milk by the FIA system using in-line digestion and the reference method.

Samples	Ref. Method (mg/L)	FIA/in-line digestion (mg/L)	Rel. deviation (%)
1	764	753	-1.4
2	659	679	+3.0
3	643	673	+4.7
4	578	542	-6.2
5	792	752	-5.1
6	720	706	-1.9
7	858	844	-1.6
8	712	698	-2.0
9	665	647	-2.7
10	674	667	-1.0
11	669	655	-2.1
12	692	665	-3.9
13	796	806	+1.3
14	823	806	-2.1

tage of lower power requirements in relation to the use of microwave digestion previously reported for other matrices (WILLIAMS et al., 1993), making this proposed approach more suitable for on-line monitoring applications. Making a determination in a complex matrix such as milk, the system saves considerable time and provides good quality results with sampling rates of about 10 determinations per hour.

Although with the off-line digestion FIA methodology smaller relative deviations (FIA vs reference method) and higher determination rates (about 30 per hour) were achieved than with the in-line digestion manifold, in the latter the entire procedure (from digestion to determination) was concluded in about six minutes, compared to more than three hours required for preparing the sample in the former.

The results obtained in this work with the UV/thermal digestion method indicate that it could be applicable to the total phosphorus determination in other complex matrices as well as to the determination of other compounds in milk, like total nitrogen.

ACKNOWLEDGEMENTS

S.M.V. Fernandes acknowledges the financial support from FCT (grant BPD/22028/99).

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Paper received May 31, 2001 Accepted August 6, 2001