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Automatized flow-batch method for fluorescent determination of free glycerol in biodiesel samples using on-line extraction

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ABSTRACT

An automatic method, based on flow-batch (FB), for determining glycerol in biodiesel was developed. The FB systems draw upon the useful features of flow, batch and multi-commutation approaches. The standards and samples preparation, as well as, derivatization and analysis were fully automated. For that purpose, a homemade chamber was built. The proposed method is based on liquid–liquid extraction of glycerol and simultaneous oxidation with periodate, generating formaldehyde that reacts with acetylacetone. A fluorescent product of 3,5-diacetyl-1,4-dihydrolutidine was obtained. The fluorescence signal was recorded at λ_{ex} = 417 nm and λ_{em} = 514 nm.

A linear response was observed from 0.10 to 5.00 mg L^{-1} glycerol, variation coefficient 1.5%, sampling rate 14 h^{-1} and detection limit 0.036 mg L^{-1} glycerol. The procedure was successfully applied to the analysis of biodiesel samples, and the results agreed with the reference method (ASTM D6584-07) at 95% confidence level.

1. Introduction

Most of the energy consumed in the world comes from fossil fuels (oil, coal and natural gas). In recent years, the energy trend is to use renewable sources in order to replace, fully or in part, fossil fuels. Among biofuels, biodiesel is one of the best alternative fuels. Biodiesel is biodegradable and nontoxic, and has low emission profiles as compared to petroleum diesel.

In recent years, biodiesel production in Argentina has been growing. Currently, Argentina ranks as the world's third largest biodiesel producer; the ranking is Malaysia, Indonesia, Argentina, USA, and Brazil. Argentina and Brazil are among the top palm and soybean growers, the two most prevalent oilseed crops in the world [1].

Biodiesel is produced by a chemical transesterification reaction, which is a sequence of three reversible chemical reactions to turn triglyceride molecule into diglyceride, monoglyceride and glycerol [2]. Consequently, two phases are obtained; the upper phase that has the main biodiesel product, and the lower phase that consists of glycerol and many other chemical compounds [3]. After the transesterification process, free glycerol can be easily removed from the biodiesel by successive washing of the biodiesel with distilled water. On the other hand, a low content of glycerides can only be achieved by selecting the appropriate reagents and reaction conditions, or by further distillation of the product [4].

The commercial biodiesel has residual amounts of glycerol that could cause occlusion of fuel filters, thus impairing the engine, causing fuel tank damage, and possibly releasing acrolein into the environment. This takes place when fuel burning occurs at a temperature above 180 °C [5–7]. International regulations specify a limit for free glycerol contents of 0.020% (w/w) [8]. In fact, biodiesel European norm EN 14214 limits the presence of some pollutants; among them are methanol and free glycerol.

In the bibliography there are several analytical methods for determining free glycerol in biodiesel. Some of them are the chromatographic methods [9,10]. Darnoko et al. [11] studied the simultaneous analysis of transesterification reaction products (triglycerides, diglycerides, monoglycerides, methyl esters, and glycerol) using gel permeation chromatography. Arzamendi et al. [12] developed the size exclusion chromatography (SEC) method for monitoring glyceride and FAME (fatty acid methyl ester) in biodiesel that was obtained from sunflower oil. Santori et al. [13] published the determination of mono-, di- and triglycerides, methyl esters, methanol and glycerol in liquid–liquid phase equilibrium, biodiesel-rich upper phase and a glycerol-rich lower phase. Reversed-phase liquid chromatography (RP-LC), in isocratic and gradient elution with UV detection was used. Another reported method was based on the gradient RP-LC (water and acetonitrile)

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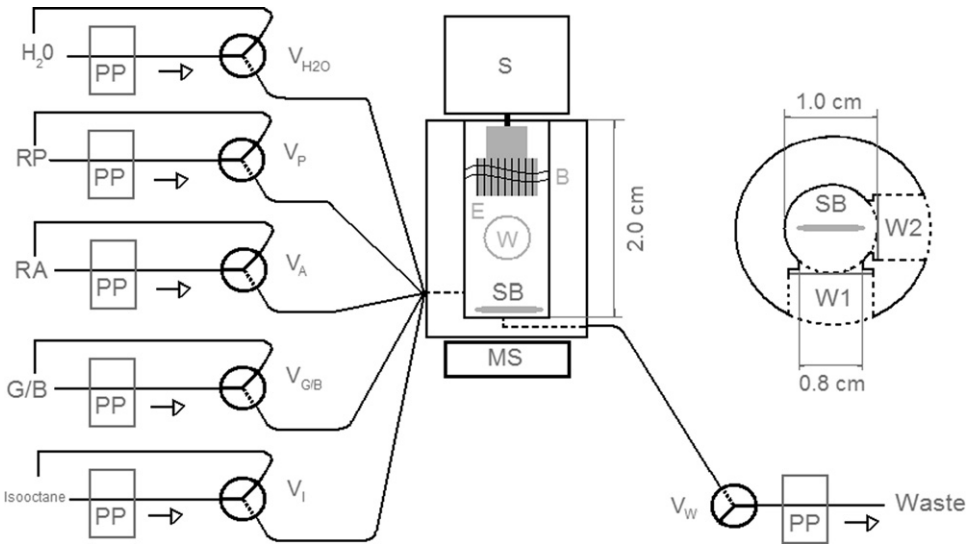

Fig. 1. Schematic diagram of the flow-batch system for free glycerol fluorescence determination. V: three-way solenoid valve; PP: peristaltic pump; S: sample; H_2O: water; RP: 15 mmol L^{-1} potassium periodate in 0.80 mol L^{-1} ammonium acetate, pH 4.5; RA: 1.50 mol L^{-1} acetylacetone in 0.80 mol L^{-1} ammonium acetate, pH 4.5; B: biodiesel phase, E: extractive solution, S: stirrer, MS: magnetic stirrer, SB: stirrer bar. W1 and W2: quartz windows, G/B: glycerol standard/biodiesel sample.

with refractometric detection. The analysis is easy and the samples do not need any treatment (only dilution by water). In addition, it has a low detection limit [14].

Dorado et al. [15] reported that the use of NIR technology provides a trustworthy and low-cost method to determine the presence of undesirable amounts of methanol and glycerol. Gonçalves-Filho and Micke [16] have developed a capillary electrophoresis methodology for determining free glycerol in biodiesel using oxidative cleavage with periodate.

Pinzi et al. [17] has used an automated on-line ultrasound assisted approach based on determining free and bounded glycerol; the reaction product was measured by spectrophotometry, at 410 nm. Bondioli and Della Bella [18] proposed a spectrophotometric measurement at 410 nm, based on periodate oxidation of glycerol, leading to the preparation of formaldehyde, on reaction with acetylacetone (Hantzsch's reaction). Silva et al. [6,19] used this analytical method in a flow multicommutation system with spectrophotometric and fluorimetric determinations of free and total glycerol. An alternative extraction procedure was also proposed to avoid the use of organic solvents, by mixing the sample and water in orbital platform for 30 min. The mixture was then centrifuged for 5 min, the biodiesel phase was removed with a Pasteur pipette and the free glycerol content was determined in the aqueous phase.

The aim of the present work was to develop an automatic method, based on flow-batch (FB), for determining glycerol in biodiesel. The FB systems draw upon the useful features of flow, batch and multi-commutation approaches. Such hybridization retains the reliability of classical batch mode methods with a modern, fully computer-controlled and miniaturized mixing assembly accessory, exchanging the use of large amounts of solutions for micro-volumes, typically employed in conventional flow systems. FB systems differ from conventional approaches in that the carrier flow is not used to transport the sample to the detector [20–28]. Moreover, the selection of washing time, flow-rates, tube diameters and lengths, is not a critical factor. The method developed by us proposes to be carried out in three stages, an on-line liquid–liquid separation of glycerol from biodiesel in the aqueous phase, a derivatization reaction in order to obtain a fluorescent product and the fluorescence detection inside the FB chamber. In order to use a fluorescent detection in the FB system, a lab made PTFE chamber with quartz windows was built.

2. Experimental

2.1. Apparatus

A fluorescence spectrometer (model FP6500, Jasco) equipped with a xenon discharge light source (150 W) was used to carry out the measurements. The slits can vary from 1 to 20 nm. The selected ones were 5 nm for excitation and 10 nm for emission.

A peristaltic pump Gilson Minipulse 3 M312 was used. This pump has a 8-channel pump head.

Gas chromatography Agilent Technologies 6890 GC with flame ionization detector (FID), programmed temperature vaporising (PTV) inlet (CIS 4, GERSTEL) and Dual Rail MPS 2 robotic sampler with 10-μL on-column syringe and an 80-μL sideport syringe with diluter module (GERSTEL) were used. Also, an Rtx-Biodiesel TG Restek column (10 m × 0.32 mm i.d.) was used [29].

2.2. Reagents, solutions and sample

All the reagents were of analytical grade chemicals, and double distilled deionized water (18 MΩ cm^{-1}) was used. Isooctane (Anedra) was used to clean the chamber.

A stock solution of 0.1 mol L^{-1} potassium hydrogen phthalate (Sigma) was prepared adjusting pH from 2.2 to 3.2 with 0.1 mol L^{-1} hydrochloric acid (Anedra).

0.2 to 1.0 mol L^{-1} ammonium acetate (Sigma) were tested adjusting pH into the range 3.2–7 with 0.1 mol L^{-1} acetic acid (Anedra).

A 1.50 mol L^{-1} acetylacetone (2,4-pentanedione, Hopkin & Williams) and 15 mmol L^{-1} potassium periodate (Analar) solutions were prepared daily in 0.80 mol L^{-1} ammonium acetate, pH 4.5. Stock solution of 10.00 mg L^{-1} glycerol was prepared by diluting glycerol (Mallinckrodt) in water.

The soybean biodiesel samples were purchased from Petrobras refinery (Dr. Ricardo Eliçabe) located in Bahía Blanca City, Argentina.

2.3. Flow-batch system

A flow-batch system was developed for extracting and determining free glycerol in biodiesel samples. A schematic diagram of

Table 1
Operation of the flow-batch system for extraction and determination of free glycerol in biodiesel.

Step	Event	Time (s)	Volume (μL)	Pump rotation speed (rpm)	Pump tube (mm i.d.)
1	Biodiesel ($V_{G/B}$)	1.0	14.8	10.0	1.29
	Potassium periodate (V_P)	0.5	7.3	10.0	1.29
	Acetylacetone (V_A)	0.5	7.3	10.0	1.29
	Water (V_{H_2O})	3.8	970.6	48.0	2.06
2	Stirrer time[a]	240	–	0.0	–
3	Detection	1.0	–	0.0	–
4	Waste (V_W)	4.2	–	48.0	2.06
5[b]	Isooctane (V_I)	1.0	238.1	48.0	2.06
6[b]	Water (V_{H_2O})	2.9	690.5	48.0	2.06
7[b]	Waste (V_W)	4.2	–	48.0	2.06
8[b]	Water (V_{H_2O})	3.9	1000.0	48.0	2.06
9[b]	Waste (V_W)	4.2	–	48.0	2.06

[a] Extraction time of the free glycerol in biodiesel.
[b] Chamber cleaning.

the proposed FB is shown in Fig. 1. The homemade FB chamber was built in PTFE with a fixed stirrer on the top, which is turned by a stepper motor (motor MDN3, 200 rpm, 9 V DC) controlled by the computer. The chamber also has a magnetic stirrer on the bottom. Additionally, two quartz windows were mounted at 90° from each other (1 cm optical path). These windows were placed in a position such that the fluorescent signal comes only from the aqueous phase containing the extracted glycerol. For all studies, a volume of 1.0 mL was used. The FB chamber was placed instead of the cuvette of the fluorescence spectrometer.

The flow system consists of six three-way solenoid valves ($V_{G/B}$, V_P, V_A, V_{H_2O}, V_W, V_I) model 161T031, NResearch, polyethylene tubing connectors with 0.8 mm id, a peristaltic pump (model M312, Gilson). The activation times of the solenoid valves ensure the reproducibility of added volumes with a standard deviation no greater than 0.03%.

A PC microcomputer connected with an interface (USB6009, National Instruments) was used to control the flow-batch system. The FB controlling software was developed in LabVIEW 7.1 (National Instruments). The same microcomputer was used to command the spectrofluorometer software.

In the flow batch system, the volume released to operate the valves was collected and weighed on an analytical scale.

Fig. 2 shows the masses of water and biodiesel at different valve activating times.

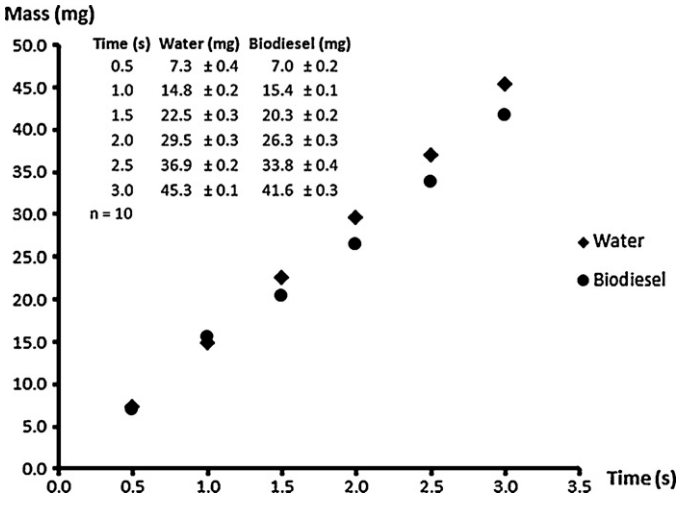

Fig. 2. Study for the action time of valves, related to the weight of the solution entered into the system. $n = 10$ replicates.

2.4. Flow-batch procedure

The procedure carried out by the FB system is shown in Table 1. To obtain the calibration curve, the standard solutions were prepared inside the FB chamber. For that purpose glycerol stock solution, potassium periodate, acetylacetone were pumped simultaneously at 10 rpm and water at 48 rpm. The final volume for each standard was maintained constant in the chamber controlling the valves activation time.

The extraction of glycerol from the biodiesel samples, previously homogenized, was carried out with 0.0154 g in 0.985 mL water inside the flow-batch chamber. The reagents consumption was 1.10 mg of acetylacetone and 25.20 μg of potassium periodate generating 1.00 mL of waste per determination.

While the solutions were pumped into the chamber both stirrers, the upper and the lower, were spinning and causing a vortex inside the chamber. This allowed the removal of glycerol from biodiesel. Therefore, it is important to highlight that the velocity of 200 rpm, and not greater, during the 4 min of extraction, was needed to prevent the development of emulsion of the biodiesel. Then the stirrers were stopped and the fluorescence signal was recorded at $\lambda_{ex} = 417$ nm and $\lambda_{em} = 514$ nm. A wash cycle, with isooctane, should be performed after each measurement.

2.5. Chromatographic procedure

The chromatographic procedure was carried out according to reference method (D6584-07 "Standard Test Method for the Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography") [29]. The peak of free glycerin was well resolved and tailless, with a retention time of 4.1 min. Each sample solution was injected by triplicate and the concentrations were calculated from a calibration curve.

3. Results and discussion

3.1. Optimization

The determination method is based on the oxidation of glycerol [30] with potassium periodate. The formaldehyde produced reacts

Table 2
Selected parameters of the FB procedure for free glycerol determination in biodiesel.

Variables	Conc. range studied	Optimum value
Periodate	0.05–0.5 mmol L^{-1}	0.11 mmol L^{-1}
Acetylacetone	1.5–15.0 mol L^{-1}	11.0 mol L^{-1}
Ammonium acetate	0.2–1.0 mol L^{-1}	0.8 mol L^{-1}
pH	2.2–7.0	4.5

Table 3
Comparative extraction procedure from different methods.

Step	Extraction of free glycerol	Time extraction	Recovery (%)	Ref.
1	1 g of biodiesel (4 mL hexane and 4 mL 50% v/v ethanol, vortex)	5 min	91–100	[18]
2	Centrifuge (2000 rpm)	15 min		
3	Remove the main part of the upper layer using a Pasteur pipette	–		
4	Take 0.5 mL of aqueous phase and 1.5 mL working solution and 1.2 mL of a 0.2 M acetylacetone solution (water bath thermostated at 70 C)	1 min		
1	200 mg of biodiesel (800 mg water and 200 μL chloroform, vigorously shaken)	10 min	95–102	[17]
2	Centrifuge (2000 rpm)	15 min		
3	Take 300 μL of aqueous phase and 300 μL solution with sodium periodate 900 mg L^{-1} spiked with internal standard	–		
1	Simultaneous oxidation-reaction with meta periodate in acceptor phase. The aqueous phase solution of sodium meta periodate and ethanol extractant mixture was used.	–	78–112	[13]
1	1 g of biodiesel (4 mL deionized water) (vortex) or (orbital platform)	5–30 min	97–115 [6]	[6,19]
2	Centrifuge (3000 rpm)	5 min	98–117 [19]	
3	Remove with using a Pasteur pipette	–		
1	15.4 mg of biodiesel (0.97 mL deionized water (stirrer))	240 s	100–103	This method

Table 4
Comparative parameters from different methods.

	BATCH [18]	FIA [13]	MC [6]	MC [19]	FB
Detection limit (mg L^{-1})	–	7	1	0.5	0.036
Sampling rate (h^{-1})	–	9	34	35	14
Effluent volume (ml)[a]	8.0	6.0	3.5	–	1.0
Extraction	Offline	Offline	Offline	Offline	Online
Extraction volume (ml)	4.00	4.00	4.00	4.00	0.9850
Sample (mg)	1000	470	1000	1000	15.4

[a] Amounts per determination.

with acetylacetone, at a suitable pH, to obtain a fluorescent product that is detected at 514 nm.

Thus, the optimization of the FB system was carried out by the univariate method. The variables optimized are shown in Table 2. These optimum values were selected as a compromise between sensitivity and reproducibility of the analytical signal. In order to corroborate that glycerol extraction was effective, a sample of biodiesel was used. This sample has been analyzed by the reference method and its concentration of free glycerol was 10.32 mg kg^{-1}. This sample was used in the FB system at different stirring times. Fig. 3 shows the obtained results. The stirrer time of 240 s was selected because at higher times no better results were obtained.

In order to check that the extraction of glycerol from the biodiesel was complete, the FB system was tested by preparing a glycerol solution on line (10.32 mg kg^{-1}), with the same concentration as the biodiesel sample. A similar fluorescence signal was obtained.

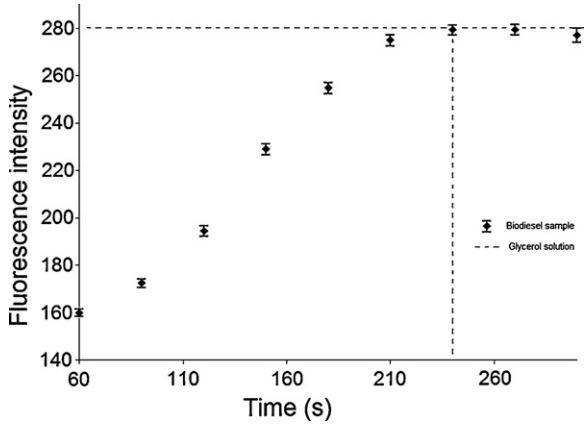

Fig. 3. Study for glycerol extraction time in a real sample (representative sample compared with a same concentration standard's signal, standard solution 10.32 mg kg^{-1} glycerol equal to 280 fluorescence intensity).

Table 3 shows some results obtained from literature on extraction methods for the analysis of free glycerol in biodiesel samples. The methods in water extraction were studied by Silva and co-authors using spectrophotometric [6] and fluorimetric [19] determination. If we compare these results with the ones obtained in the present work, it can be observed that the results did not differ significantly from those obtained with organic solvents. But the most significant improvement of this study is the on-line extraction and the reduced extraction time.

Table 5
Recovery study.

Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
0[a]	0.98 1.01 0.99	
0.25	1.25 1.24 1.24	100 ± 3
0.75	1.75 1.75 1.77	102 ± 1
1.25	2.26 2.25 2.27	101 ± 1
2.5	3.54 3.56 3.57	103 ± 0.4
3.75	4.78 4.76 4.80	101 ± 1

[a] Sample 1.

Table 6
Commercial samples to validate the method.

Sample	Reference method			Proposed methods			% error (ppm)
	mg kg^{-1} glycerol	ppm	SDV[a]	mg kg^{-1} glycerol	ppm	SDV[a]	
1	3.9	0.98	0.04	4.0	1.00	0.02	2
2	15.4	3.84	0.02	15.2	3.80	0.04	4
3	8.0	2.00	0.02	7.9	1.98	0.01	2
4	10.2	2.54	0.03	10.3	2.58	0.02	4
5	5.0	1.25	0.01	4.9	1.23	0.01	2

[a]The samples were analyzed by triplicate.

3.2. Analytical performance

By using the proposed flow-batch system, the calibration curve was $I = 105.410\ (\pm 0.822) + 67.687\ (\pm 1.522)C$ (mg L^{-1}), $r = 0.998$. Under the selected conditions, a linear response was observed within the range 0.10–5.00 mg L^{-1} glycerol, which is equivalent to 0.40–20.00 mg kg^{-1} free glycerol in biodiesel. The detection limit was estimated at 0.036 mg L^{-1} with 95.0% of confidence level, and quantification limit was estimated at 0.121 mg L^{-1} for the same confidence level, calculated from the calibration curve [31]. The detection limit is 1389 times (equivalent to 0.144 mg kg^{-1} in biodiesel) lower than the threshold value established by European, American and Brazilian regulations [8]. The relative standard deviation (RSD%) was 1.5% and it was obtained from 5 replicates of real samples of 3 mg L^{-1} free glycerol concentration. The sample throughput was estimated at 14 samples per hour.

Table 4 compares the analytical features of the proposed and bibliography procedures. The proposed flow-batch system has the following advantages: it includes online liquid–liquid extraction and it has a lower detection limit (LOD, 0.036 mg L^{-1}). It was observed that the LOD was 28 and 14 times lower than that reported in previous multicommutation systems [6,19]. Further advantages are the sample amount (15.4 mg), which is 30.5 and 65 times smaller in the consumption of biodiesel than in the FIA [13] and multicommutation [6,19] methodologies, respectively. The possibilities of flow-batch provide the option to take less amount of sample. Two other advantages include waste disposal (1.0 mL), and, finally, the sampling rate (14 h^{-1}) which is close to that in the FIA procedure.

3.3. Validation

3.3.1. Recovery study

A recovery study was also performed. For that purpose, an appropriate amount of the samples was weighed, spiked with different amounts of glycerol, and analyzed with the flow-batch method. Table 5 shows the results obtained. Satisfactory percentage recoveries were obtained for this kind of fuel.

3.3.2. Real samples

The accuracy of the method was validated by comparison with the reference method (Gas Chromatography) [29]. Table 6 shows the results of the validation. Notably, for all analyzed samples, the concentrations of free glycerol in the biodiesel obtained by our proposed method agreed with those obtained by GC. The recovery values ranged between 100 and 103% for our proposed method. In all cases, SDV (standard deviation of mean) values were lower than 0.04%.

In order to obtain further understanding of the accuracy ability of both methods, the reference method and the proposed method, a regression line was obtained. The estimated intercept (a) and slope (b) were compared with their ideal values of 0 and 1 using the elliptical joint confidence region (EJCR) test [25,32,33]. The intercept $-(0.09 \pm 0.008)$ and the slope (0.99 ± 0.010) values demonstrated that the joint confidence region certainly contains the theoretical (0, 1) point ($\alpha = 0.05$, 2, n-2 = 8). The proposed procedure agrees with those obtained in the reference one.

4. Conclusion

The proposed flow-batch method is fully automated, because it is possible to do the extraction of free glycerol, derivatization of chemical reaction to obtain the product and record the fluorescence spectrum in the chamber. The addition of reagents and the different samples, the stirring and recorded signals are handled by the computer through LabView software.

The results obtained by the proposed flow-batch system have been better than those from bibliography. The proposed method's advantages of automation are many; such as the decrease in reagent consumption, waste volume (environmentally friendly), as well as LOD improvement.

The automation makes it possible to facilitate the analytical determination, being able to obtain a fast and simple method, which is very important in implementing routine jobs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.10.055.

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