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Spectrophotometric determination of paracetamol using hydrogel based semi solid-liquid dispersive microextraction

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Abstract. A simple, fast and inexpensive method based on Dispersive Liquid-Liquid Microextraction (DLLME) and Dispersive Solid-Phase Extraction (DSPE) has been developed for preconcentration and spectrophotometric determination of paracetamol (PC) using pH-sensitive hydrogel (HG). In this study, we demonstrate a novel microextraction technique, entitled "semi solid-liquid dispersive microextraction", which can be successfully used for preconcentration of analytes of interest from aqueous samples. The procedure involves the oxidation of paracetamol by Fe(III) and a subsequent reaction with ferricyanide in the presence of HCl to yield a Prussian Blue (PB) complex. In the extraction step, an appropriate amount of poly(styrene-alt-maleic anhydride), as a pH-sensitive hyrogel, was injected into the aqueous solution, so a cloudy solution was formed. Organic and inorganic compounds, having the potential to interact with polymer particles, could be extracted into the organic phase. For our case, the PB complex could be extracted to the cloudy phase. After centrifuging, the hydrogel-rich phase was sedimented at the bottom of the centrifuge tube. The absorbance of the sedimented phase diluted in methanol was measured at the absorption maximum of 733 nm (λ_{max} of PB in hydrogel). The absorbance was linear to paracetamol concentration in the range of 0.01-0.5 μg mL^{-1}, with a correlation coefficient of 0.995.

1. Introduction

Sample preparation, including both clean-up and the preconcentration, is usually the most important and crucial step in the whole analytical process, because it helps to achieve lower detection limits. Traditional Liquid-Liquid Extraction (LLE) is widely employed for sample preparation [1]. However, some shortcomings, such as lengthy processing time and the use of large amounts of toxic organic solvents, can limit the success of LLE in sample preparation. Solid-Phase Extraction (SPE) methodologies offer high extraction recoveries and use much less solvent than LLE. However, their selectivity is relatively low and an extra step of concentration is needed [2]. Solid-Phase Microextraction (SPME) is a solvent free technique, which includes simultaneous extraction and preconcentration of analytes from aqueous samples or the headspace of the samples [3]. SPME is a simple, portable and fast method; however, cost, a limited lifetime and the fragility of fibers can be a problem. Cloud Point Extraction (CPE) is an outstanding alternative for microcomponents preconcentration, which has been developed in recent years [4-7] because it provides high preconcentration efficiency, and uses inexpensive and non-toxic reagents. However, surfactants used as

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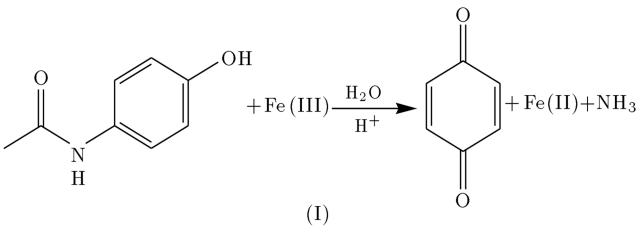
extracting agents in the CPE often need salt addition, temperature control and pH adjustment [8-10]. Dispersive Liquid-Liquid Microextraction (DLLME) was introduced by Assadi and co-workers in 2006 [11]. It is a simple and fast method based on the use of an extractant, i.e. a few microliters of an organic solvent with high density and a disperser solvent with high miscibility in both extracting and aqueous phases. When the mixture of the extracting phase and disperser is rapidly injected into the sample, a cloudy solution is formed. Since the surface area between the extracting solvent and the aqueous sample becomes very large, equilibrium state is achieved quickly and, therefore, the extraction is independent of time. In fact, this is the main advantage of DLLME. Despite the mentioned advantages, this method suffers from some deficiencies. Highly toxic and chlorinated solvents are usually employed as extractants in DLLME. Also, applying a higher density of extracting solvents leads to some problems, such as the incompatibility of the extracting solvents with some instruments, such as ICP-OES and reverse-phase HPLC [12]. In addition, DLLME is not a selective extraction technique [13]. Dispersive Solid-Phase Extraction (DSPE), often referred to as the "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, was first introduced by Anastassiades et al. in 2003 [14]. The method is widely used for multi-residue analysis of multiclass pesticides [15-17]. In order to clean-up the crude extract, a small amount of SPE sorbent material was added to an aliquot of the extract, thereby, matrix co-extractives can be removed. The clean-up is easily carried out by just shaking and centrifuging [18-20]. The main disadvantage of the QuEChERS is its lower extracted concentration (nearly 2-5 times) compared to most other traditional methods [21].

The proposed method is an efficient, rapid, simple and inexpensive microextraction technique based on a combination of Dispersive Solid-Phase Extraction (DSPE) and Dispersive Liquid-Liquid Microextraction (DLLME) methods. The method, entitled "semi solid-liquid dispersive microextraction", is developed as a new analytical approach for extracting, cleaning up and preconcentrating analytes of interest from aqueous samples. This method offers the advantages of both dispersive liquid-liquid microextraction and solid-phase extraction methods. The surface area between the extracting solvent and the aqueous sample becomes very large, so, the equilibrium state is achieved quickly and the extraction time is very short. On the other hand, the high density gel provides easy phase separation. It is worth noting that this method overcomes the disadvantages of the mentioned methods; there is no need for using extraction and dispersive organic solvents compared to the conventional DLLME and, also, recovery problems of adsorbed analytes in the sorbent-desorption step are omitted compared to the DSPE. This procedure integrates sampling, extraction and concentration into a single solvent-free step.

Stimuli responsive polymers or hydrogels are materials with three dimensional networks and high-molecular weight. Hydrogels behave like solids, due to a three-dimensional cross-linked network within the liquid, and can change their volume significantly [22]. These polymers are intended to function as a platform technology for the delivery of many types of biomolecule. Interest in hydrogels has gained momentum recently, because these materials can response to small alterations of certain environmental parameters: pH [23], ionic strength, salinity [24], electrical current [25] and temperature [26]. pH-sensitive polymers are synthesized by putting acidic or basic functional groups on the polymer structure; the substituted groups being able to exchange protons in an aqueous media. Organic or inorganic compounds having the potential to interact with polymer particles in the solution (chemical interaction or physical adsorption) may be extracted.

Determination of paracetamol in pharmaceuticals is of paramount importance, as paracetamol overdose is a frequent cause of fulminating hepatic failure and other toxic effects. Various analytical methods have been reported for the determination of paracetamol, in pure form, formulation, and in pharmaceutical and biological samples. Several published methods for paracetamol determination include: spectrophotometric methods [27-30], fluorimetry [31], Flow-Injection Analysis (FIA) [32,33], FT-IR spectrophotometric methods [34-36], various modes of electrochemistry [37-39], High Performance Liquid Chromatography (HPLC) [40,41], High Performance Liquid Chromatography/Supercritical Fluid Chromatography (HPLC-SFC) [42], Thin-Layer Chromatography (TLC) [43] and also capillary electrophoretic methods have been proposed for the determination of paracetamol [44]. Hydrogels for preconcentration and extraction purposes have not been used. Recently, a novel and sensitive cloud point extraction method using pH-sensitive hydrogel for the preconcentration and determination of trace amounts of malachite green (which is a water pollutant compound) in water samples has been developed [45]. The method is based on extraction of the analyte mediated by pH-sensitive hydrogel poly(styrene-alt-maleic anhydride). Simplicity of operation, rapidity and low cost are the advantages of this extraction method. The aim of this work is to extend the applicability of the proposed method in the sample preparation to more complex matrix samples. In the present work, semi-solid-liquid dispersive microextraction was explored for the extraction of paracetamol in serum samples. A pH-sensitive hydrogel was employed for the preconcentration and



$$Fe(II)+K_3[Fe^{III}(CN)_6] \longrightarrow KFe^{III}[Fe^{II}(CN)_6] \quad (II)$$

Scheme 1. Reaction mechanism.

determination of paracetamol in blood serum samples. The method is based on formation and application of PB complex. The reaction takes place in two steps: (I) oxidation of paracetamol by Fe^{3+}, and (II) the subsequent reaction of in situ formed Fe^{2+} with potassium hexacyanoferrate(III) [46]. The proposed mechanism is presented in Scheme 1. The formed complex is highly insoluble ($K_{sp} = 3 \times 10^{-41}$) [47]. The PB complex is extracted by pH-sensitive hydrogel (poly(styrene-alt-maleic anhydride)). After centrifuging, the sedimented phase was diluted in methanol and the absorbance was measured at 733 nm.

2. Experimental procedure

2.1. Reagents and solutions

All reagents were prepared from analytical grade reagents. Paracetamol, potassium ferricyanide, ferric nitrate, styrene, maleic anhydride, benzoyl peroxide, tetrahydrofuran, diethyl ether and NaOH were provided by Merck (Darmstadt, Germany). HCl was purchased from Aldrich (St Louis, US). Poly(Styrene-alt-Maleic Anhydride) (PSMA) (120,000 average MW) was synthesized through free-radical polymerization of styrene and maleic anhydride followed by hydrolysis to form poly(styrene-alt-maleic acid) that incorporates two carboxylic groups and a phenyl group in each repeating unit [48]. This product is also commercially available (Sigma-Aldrich, Product no. 662631).

A standard paracetamol stock solution (50 μg mL^{-1}) was prepared by dissolving an appropriate amount of paracetamol in distilled water (5 mg/100 mL). By further dilution, the working standard solution of the drug (1 μg mL^{-1}) was obtained. The potassium ferricyanide solution (0.0329 g, 0.1 mmol), ferric nitrate (0.404 g, 1 mmol) and HCl (1 mol L^{-1}) were prepared in distilled water (50 mL). An aqueous solution of hydrogel (0.2 g, 0.4% w/v) was prepared by dissolving the polymer in water (50 mL) in the presence of aliquots of NaOH (0.1 mol L^{-1}). A blood sample was taken from a healthy volunteer, centrifuged at 3000 rpm for 10 mins, and serum fractions were collected. Precipitation of proteins was carried out by adding acetonitrile (1:1, v/v) to the serum sample. The tube was centrifuged and the supernatant liquid was used. The sample was previously 50 times diluted, and it is assumed that the paracetamol concentration of blood samples is zero. Serum samples were spiked with appropriate amounts of standard solutions in paracetamol; concentration ranges from 0 to 0.5 μg mL^{-1}.

2.2. Preparation of poly(styrene-alt-maleic acid)

Poly(Styrene-alt-Maleic Anhydride) (PSMA) was prepared from styrene and maleic anhydride via thermally initiated free-radical polymerization, according to the literature [49].

2.3. Sample preparation

Aliquot portions of paracetamol standard solution were transferred into 10 mL volumetric flasks (final concentration: 0.01 - 0.5 μg mL^{-1}); $K_3[Fe(CN)_6]$ (1.3 mL) and $Fe(NO_3)_3$ (0.8 mL) were added and the resulting solutions were mixed thoroughly. After 10 min. HCl (2 mL) was added and diluted to the volume with distilled water, and the flasks were set aside for 20 minutes. After this period of time, the samples were transported to 15 mL falcon tubes and, for dispersive microextraction, HG 0.4% (0.7 mL) was injected to the solutions. A cloudy solution was formed. The test tubes were shaken gently and after centrifugation for 10 min at 5000 rpm, the hydrogel-rich phase was made up to 0.5 mL by adding methanol. The absorbance values were measured at 733 nm against a reagent blank prepared with the same reagent concentrations. The spectra of the hydrogel rich-phase after extraction in the presence and absence of paracetamol are represented in Figure 1. The calibration curve was drawn and the regression equation was calculated.

2.4. Instruments

A model T80$^+$ UV/V is double-beam spectrophotometer with a PG mode (China) with 1-cm quartz cells

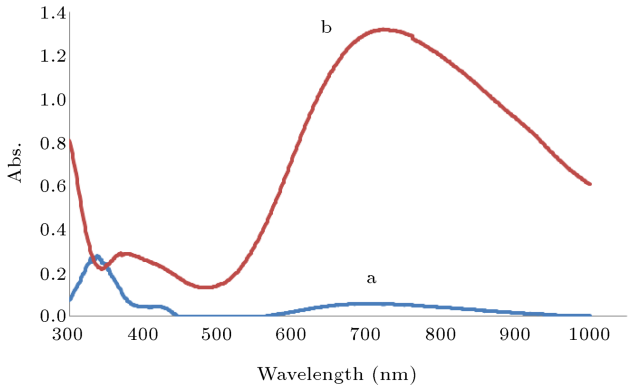


Figure 1. Absorbtion spectra of (a) hydrogel-rich phase diluted with methanol and (b) hydrogel-rich phase containing paracetamol 0.2 mg mL^{-1}.

(volume 0.5 mL) was employed for spectrophotometric measurements. A refrigerated centrifuge type Hettich 320R (Germany) was used for accelerating the phase separation process.

2.5. HPLC conditions

The HPLC method was carried out in order to determine paracetamol in serum samples. HPLC was performed with a Knauer (Berlin, Germany) system equipped with a UV Diode-Array Detector (DAD). Data processing and integration were performed with Chromgate software. Column temperature was set at 50°C by use of a Jetstream (Knauer Co.) column thermostat. Separation was achieved on a C_{18} column (250×4.6 mm i.d., 5 μm, Knauer Co.); a mobile phase, composed of water and methanol (70:30), flow rate: 1.5 mL/min, and wavelength of detection: 245 nm.

2.6. Statistical software

An MS Excel add-in software: essential regression and experimental design for chemists and engineers (EREGRESS) [50,51] was used to design the experiments, and model and analyze the results.

2.7. Central composite design

Identification of significant variables in the preconcentration of paracetamol was performed by a Central Composite Design (CCD). Four independent variables, namely, a concentration of ferricyanide (F_1), iron(III) nitrate (F_2), HCl (F_3) and hydrogel (F_4), were studied at five levels with four repeats at the central point using a circumscribed design. According to the central composite design, 28 experiments were generated in random order, including a 2^4 of the two-level factorial design, central points, and star points. Levels of independent parameters were determined based on preliminary experiments. For each of the four studied variables, high (coded value: +2) and low (coded value: -2) set points were selected to construct a rotatable design, as shown in Table 1. A list of experiments based on the CCD methodology (coded values), and the response of each run, which have been achieved using EREGRESS software, are represented in Table 2.

A second-order polynomial equation was used to express absorbance as a function of independent

Table 2. Central Composite Design (CCD) with four independent variables (coded values), and the responses.

Design points	Factor levels				Response
	F_1	F_2	F_3	F_4	
1	-1	1	-1	-1	0.616
2	0	0	2	0	0.794
3	-1	-1	1	1	0.626
4	1	1	-1	-1	0.602
5	1	1	1	1	0.882
6	0	0	0	-2	0.420
7	1	1	-1	1	0.908
8	-1	1	1	1	0.696
9	-1	-1	-1	-1	0.520
10	2	0	0	0	0.960
11(cp)*	0	0	0	0	0.770
12	1	-1	-1	1	0.746
13	0	0	-2	0	0.730
14	-1	1	-1	1	0.554
15(cp)	0	0	0	0	0.840
16	-1	-1	-1	1	0.610
17	1	-1	1	1	0.804
18(cp)	0	0	0	0	0.712
19	-1	-1	1	-1	0.700
20	1	-1	1	-1	0.562
21	-1	1	1	-1	0.716
22	-2	0	0	0	0.572
23	1	1	1	-1	0.632
24	1	-1	-1	-1	0.482
25	0	-2	0	0	0.372
26	0	0	0	2	0.764
27	0	2	0	0	0.560
28(cp)	0	0	0	0	0.732

*: (cp) Indicates 4 repeats of the center point.

Table 1. The variables and values used for the Central Composite Design (CCD).

Variable name	Coded factor levels				
	-2 (low)	-1	0	+1	+2 (high)
F_1 $K_3[Fe(CN)_6]$ (mmol L^{-1})	0.06	0.12	0.18	0.24	0.3
F_2 $Fe(NO_3)_3$ (mmol L^{-1})	0.20	0.80	1.40	2	2.6
F_3 HCl (mol L^{-1})	0.08	0.11	0.14	0.17	0.2
F_4 HG (%w/v)	0.004	0.012	0.02	0.028	0.036

variables. The model, including linear, quadratic and interaction terms, can be presented as Eq. (1).

$$\begin{aligned}\text{Response} =& b_0 + b_1 \times F_1 + b_2 \times F_2 + b_3 \times F_3 \\ &+ b_4 \times F_4 + b_5 \times F_1 \times F_1 + b_6 \times F_2 \times F_2 \\ &+ b_7 \times F_3 \times F_3 + b_8 \times F_4 \times F_4 \\ &+ b_9 \times F_1 \times F_2 + b_{10} \times F_1 \times F_3 \\ &+ b_{11} \times F_1 \times F_4 + b_{12} \times F_2 \times F_3 \\ &+ b_{13} \times F_2 \times F_4 + b_{14} \times F_3 \times F_4, \qquad (1)\end{aligned}$$

where $F_1 - F_4$ are the variable parameters, and $b_0 - b_{14}$ are the coefficient values obtained through Multiple Linear Regression (MLR). Response Surface Methodology (RSM) was used to assemble the model in order to describe the way in which the variables are related and the way in which they influence the response. A central composite coupled with response surface methodology generate a mathematical model to determine the correlation between the response factor and independent variables. The response surface plots were obtained through a statistical process that described the design and the modeled CCD data. Response surface methodologies graphically illustrate relationships between parameters and responses, and are the way to obtain an exact optimum [50,51]. For testing the fitness of the regression equation, the multiple correlation coefficient (R) and the squared regression coefficient (R^2) were evaluated. The results indicate that the second order polynomial model was highly significant and adequate to represent the actual relationship between the response and the independent variables. The analysis of variance (ANOVA) and least squares techniques were used to determine adequacy between the predicted model and the experimental results. Optimal conditions were determined from the three dimensional (3D) surface plots of the response.

3. Result and discussion

3.1. Experimental design

In order to optimize four independent variables, namely, the concentration of ferricyanide (F_1), iron(III) nitrate (F_2), HCl (F_3) and hydrogel (F_4), the central composite design with a quadratic model was employed. The aims of this CCD strategy were:

(i) Investigating the effects of concentration of reagents and hydrogel on the preconcentration and determination of paracetamol;

(ii) Identification of the variables that have a higher impact on the extraction procedure;

(iii) To give an insight into the robustness of the method close to optimum conditions;

(iv) To eventually show interactions between the variables.

We started with a full quadratic model, including all terms of Eq. (1), to find the important factors. Then, in order to reach a simple yet realistic model, insignificant terms ($p > 0.05$) were eliminated from the model through a "backward elimination" process. By elimination of insignificant parameters from Eq. (1), calibration R^2 decreased finally to 0.892, but adjusted R^2 (R^2_{adj}), and R^2 for prediction (R^2_{pred}) increased nearly to 0.861 and 0.810, respectively. The main characteristics of the reduced model were within acceptable limits, which reveal that the experimental data are a good fit with the second-order polynomial equation. The obtained reduced model, including R^2 values, PRESS, standard error and significant linear, quadratic and interaction parameters, is given in Table 3.

3.2. Response surface method and selection of optimum conditions

Response Surface Methodology (RSM) is useful for modeling while the response of interest is influenced by several variables and the objective is to optimize this response. Three dimensional (3D) graphs provide

Table 3. Statistical data for the constructed model.

Regression equation	Coefficient	Value
$\text{Abs} = b_0 + b_1 \times \text{Fe(NO}_3)_3 + b_2 \times \text{HCl} + b_3$	b_0	-0.219
$\times \text{Fe(CN)}_6^{3-} \times \text{HCl} + b_4 \times \text{Fe(CN)}_6^{3-} \times \text{HG}$	b_1	0.616
$+ b_5 \times \text{Fe(NO}_3)_3 \times \text{Fe(NO}_3)_3 + b_6 \times \text{HG} \times \text{HG}$	b_2	3.765
$\lvert R \rvert$ 0.945	b_3	-15.45
R^2 0.892	b_4	156.4
R^2 adjusted 0.861	b_5	-0.197
Standard error 0.0533	b_6	-491.0
Points no. 28		
PRESS 0.110		
R^2 for prediction 0.810		

insight about the effect of each variable in order to achieve peak performance. Plots for the predicted responses were prepared based on the model function to analyze the change in response surface. Some of the response surface plots are represented in Figure 2, which illustrates the relationship between two variables and the absorbance (733 nm) of samples, while two other variables were fixed at the central point. The surface plots reveal a non-linear relation between the response and the variables, $F_1 - F_4$, because the surface plots of the response are curvature. The coefficients, shown in Table 3, show the significance of linear, quadratic, and cross terms. Using response surfaces, optimum conditions are obtained and presented in Table 4.

From the constructed models, using real factors for optimization of the variables to extract and determine paracetamol (Table 3 and Figure 2), the following results were obtained: $Fe(NO_3)_3$ (F_1) has a significant effect on the response using both linear and quadratic terms. HCl (F_2) not only has an effect on preconcentration as a linear variable, but also interacts significantly with the concentration of ferricyanide. In addition, response surface plots show that hydrogel is one of the effective parameters and has an interaction with the ferricyanide ($b_4 \times Fe(CN)_6^{3-} \times HG$). The coefficients are presented in Table 4.

3.3. Analytical characteristics

After optimization of all parameters effective on the PB reaction and extraction procedure of paracetamol, a linear relationship between the absorbance and concentration of the drug was obtained in the range of 0.01-0.5 μg mL^{-1}. The regression equation, $A = 4.342C + 0.028$ (where A = absorbance, and C = concentration in μg mL^{-1}), with a correla-

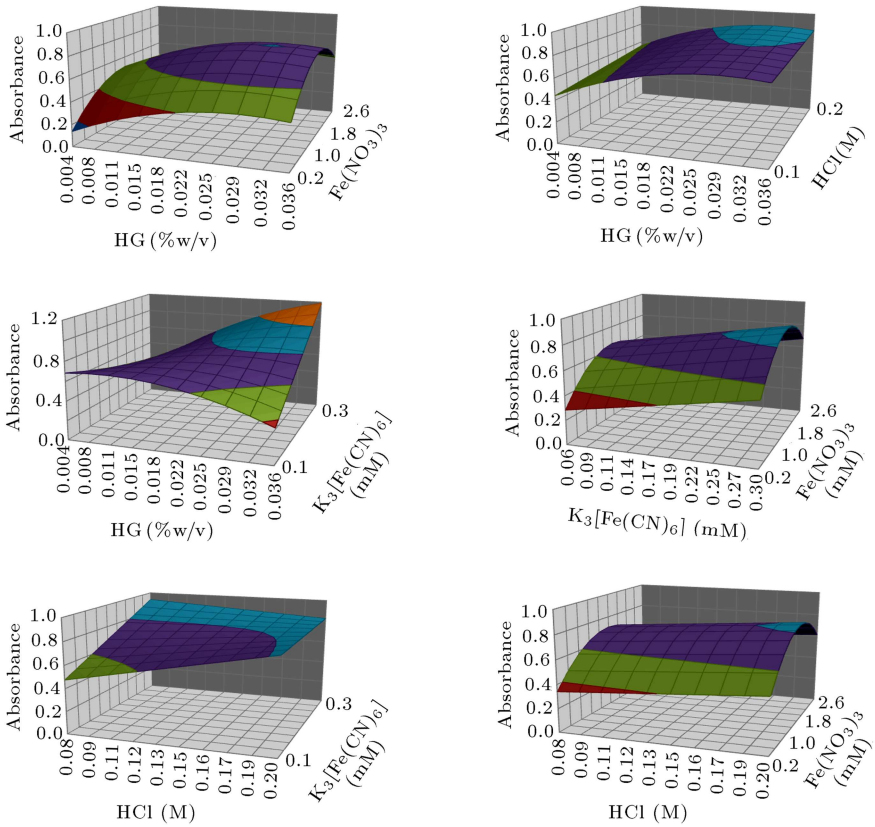


Figure 2. Response surface plots.

Table 4. Optimum conditions obtained by response surface modeling.

	Variable name	Optimum values	Selected values
F_1	$K_3[Fe(CN)_6]$ (mmol L^{-1})	0.23-0.3	0.26
F_2	$Fe(NO_3)_3$ (mmol L^{-1})	1.5-1.7	1.6
F_3	HCl (mol L^{-1})	0.18-0.2	0.19
F_4	HG (%w/v)	0.026-0.03	0.028

tion coefficient of 0.995 was obtained. The limit of detection ($3S_b/m$), concentration and improvement factors, and extraction recovery are presented in Table 5. Also, a Relative Standard Deviation (R.S.D.) of 2.6% was obtained for 7 replicate determination of 0.2 μg mL^{-1} paracetamol, according to the proposed method.

Table 5. Analytical characteristics of the proposed method.

Regression equation[a]	$Abs = 4.342C + 0.028$
Regression equation before extraction	$Abs = 0.225C + 0.005$
R^2 [b]	0.995
Linear range (μg mL^{-1})	0.01-0.5
LOD (μg mL^{-1})[c]	7×10^{-3}
Repeatability (RSD)[d]	2.6
Concentration factor	20
Improvement factor[e]	19.7
Recovery of extraction[f] (%)	98.7

[a]: Concentration of paracetamol in μ g mL^{-1};

[b]: Squared regression coefficient;

[c]: Limit of detection for S/N = 3;

[d]: Relative standard deviation for 7 replicate determination of 0.2 μg mL^{-1} paracetamol;

[e]: The ratio of the slope of the calibration graph for the microextraction method to that of the slope of the calibration graph without preconcentration;

[f]: The ratio of the improvement factor to the concentration factor, assuming equal molar absorption coefficient in the two environments.

The proposed microextraction method is compared with several previously reported techniques for the assay of paracetamol, and the results are shown in Table 6. As can be seen, semi-solid-liquid dispersive microextraction provides a satisfactory dynamic range, and also a lower or comparable detection limit compared to that of chromatographic methods.

3.4. Application of the proposed method

The extraction of the drug was conducted according to the proposed procedure by analyzing human serum samples. An aliquot of serum (2 mL) was deproteinized by a simple procedure involving the addition of 2 mL acetonitrile, vortex mixing for a few seconds, and centrifugation for 10 min [52]. 1 mL of the clear supernate was then analyzed according to the proposed method. A standard addition method was applied in order to circumvent the matrix effect. The results of several spiked samples are presented in Table 7. The recoveries are close to 100% and indicate that the method was successful. The accuracy of the determination of paracetamol in serum samples by the proposed method was also tested by analyzing the treated serum sample by HPLC (the conditions are represented at Section 2.5). An F-test was applied to detect possible differences between the results of the proposed method and the HPLC method. The imperial F-value was lower than critical value at 95% and a degree of freedom of 3,3, which admits that

Table 6. Comparison of the proposed method with diverse methods for the determination of paracetamol.

Method	Remark	Linear range	Detection limit	Reference
HPLC	Determination of the ternary combination of paracetamol, caffeine and dipyrone	0.409-400 μg mL^{-1}	0.409 μg mL^{-1}	[53]
Reversed-phase HPLC	Simultaneous determination of paracetamol and other analgesic and antipyretic drugs in pharmaceutical formulations	100-300 μg mL^{-1}	0.39 μg mL^{-1}	[54]
MIP-BAW sensor	A MIP-bulk acoustic wave sensor for paracetamol has been designed with an imprinted polymer as a sensing material	5.0×10^{-3}-0.1 μM	5.0×10^{3} μM	[55]
SPME-GC-MS	Determining trace amounts of biologically active substances in water samples	ng L^{-1} to mg L^{-1}level	10 μg L^{-1}	[56]
Cyclic voltammetery	Electrochemical oxidation of paracetamol mediated by nanoparticles bismuth oxide modified glassy carbon electrode	5.0×10^{-7}-1.5×10^{-3} M	2.0×10^{-7} M	[57]
Semi solid-liquid dispersive microextraction	Based on applying pH-sensitive hydrogel for preconcentration	0.01-0.5 μg mL^{-1}	7×10^{3} μg mL^{-1}	This work

Table 7. Recoveries of paracetamol from blood serum ($n = 3$).

Paracetamol (μg mL^{-1})		
Spiked	**Found**	**Recovery (%)**
0	< LOD	–
0.10	0.093	93.0
0.20	0.187	93.5
0.30	0.318	106
0.40	0.408	102

the proposed preconcentration method can be used for analytical purposes in the future.

4. Conclusions

This paper establishes a novel microextraction method, entitled "semi solid-liquid dispersive microextraction", based on pH-sensitive hydrogel. The proposed method provides a very simple and inexpensive procedure for preconcentration and determination of paracetamol. Utilizing hydrogel offers the advantages of liquid-liquid microextraction (DLLME) and Dispersive Solid-Phase Extraction (DSPE), and toxic solvent extraction has been avoided. Therefore, the method can be classified as a green analytical method. The results of this study clearly show the potential and versatility of this microextraction method, which was applied to spectrophotometric determination of paracetamol in blood samples.

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