Chapter 6

Diamond paste-based electrodes for the determination of L- and D-fucose using differential pulse voltammetry

6.1 Introduction

Electrochemical techniques have been developed for the analysis of trace amounts of many organic molecules of biological significance. The selection of electrode material is very crucial for the selective assay of specific group of compounds. Different materials have been used, such as glass carbon, pyrolytic graphite, and carbon paste for the amperometric electrodes design. Glassy carbon and pyrolytic graphite electrodes have got practical problems, such as electrode surface fouling due to binding of reaction products and non-electrochemically active biochemical species [1]. Diamond exhibits several unique features, which make it superior to those conventional electrode materials [2-12]. Polycrystalline boron-dopped diamond thin film electrodes have been used in electrochemical studies due to their wide electrochemical potential window allowing a sensitive electroanalytical detection of chemical species that reacts at relatively high potentials [5], low background current, long-term stability due lack of adsorption of chemical species [8] on the inert electrode surface and finally their relative insensitivity to dissolved oxygen in both alkaline and acidic aqueous electrolytes [13,14].

The advances in single-crystal diamond have enabled the development of a wide range of monocrystalline diamond products. Electrical properties of the monocrystalline diamond,

based on the mobilities of negative electrons and positive holes, the inherent structural defects and the presence of small amounts of impurities in its structure enhance its use for research in the design of electrochemical sensors [15, 16].

Differential pulse voltammetry has proved to be effective for the determination of several chiral compounds in biological, pharmaceutical and clinical matrices involving limited or no sample preparations such as the time-consuming extraction techniques. Chirality remains a vital diagnosis tool as biological marker for many clinical diseases and a key parameter in the development of pharmaceuticals and agrochemicals [17]. In many cases only one isomer in a chiral compound is responsible for the desired activity, while the other may exhibit no value or interference. Recent descriptions of diseases producing abnormal chiral metabolites have provided the need for individual enantiomer assay.

The fucose enantiomers are L- and D-6-deoxygalactoses monosaccharide (Figure 6.1). D-fucose exists in simple glycosides comprising only a few sugar units, limited to plant products, microbial and antibiotic substances [18]. The L-enantiomer occurs in many bacterial and plant glycosides and polysaccharides [18]. L-fucose is also found in oligosaccharides of human milk, and in many glycolipids and glycoproteins, including several families of blood group antigens [19]. Changes have been detected in the fucosylation pattern of these molecules in the tissues of cancers patients, due to fucosyltarnsferase activity, which is especially high in the serum of patients suffering from high malignant or metastatic tumors (such as colon carcinoma, breast and liver cancer) [20]. Serum and urinary levels of free L-fucose of cancer patients had

significantly higher levels than healthy persons [18-19]. Clinically, levels of free L-fucose in serum and urine, can be used as a marker for tumor malignancy.

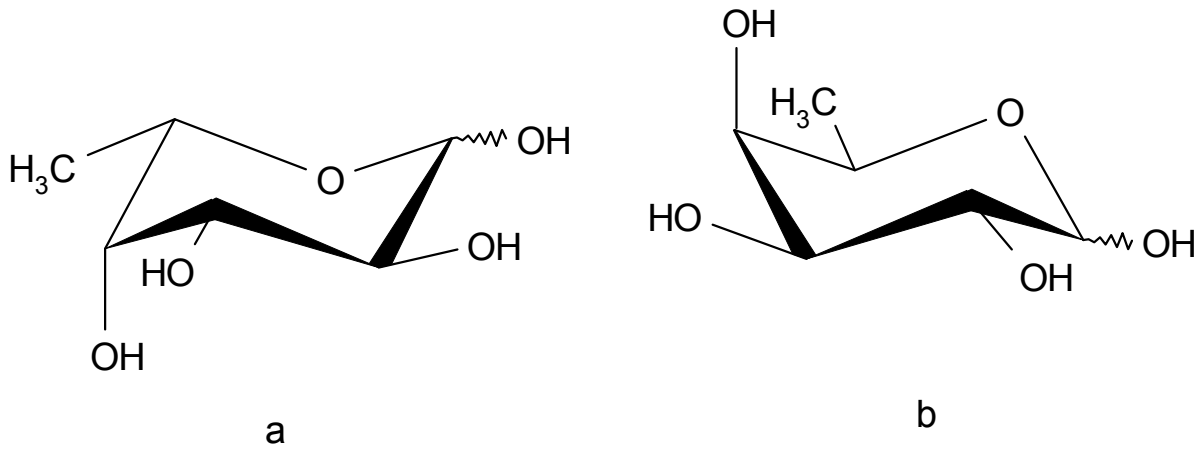

Figure 6.1. Chair structures of fucose (a) L-fucose and (b) D-fucose

Different analytical methods have been used for the assay of L- and D-fucose including enzymatic assay [21-22], gas chromatography [23], high performance liquid chromatography [24-25], anion exchange chromatography [26], and spectrophotometry [27-29].

The aim of this chapter is to develop a simple and reliable method based on utilization of diamond electrodes for the determination of L- and D-fucose in biological fluids (e.g., serum, urine). A diamond paste was preferred for the electrode design due to the high reliable construction [30]. A natural and two synthetic types of diamonds (1 and 2) were used for the design of the diamond paste.

6.2 Experimental section

6.2.1 Apparatus

A 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 100 and software (Eco Chemie version 4.9) were used for all cyclic and differential pulse voltammetric measurements. An Ag/AgCl (0.1 mol/l KCl) electrode and a Pt electrode were used as reference and counter electrodes in the cell.

6.2.2 Diamond paste electrodes design

Three diamond paste electrodes were prepared for L- and D-fucose using different diamond powder (natural diamond, synthetic 1 and synthetic 2). All diamond paste electrodes were prepared by mixing 100mg of each diamond powder with 20μL of paraffin oil. A portion of the diamond paste was then placed in a plastic pipette tip (3mm). The electrode diameter was approximately 2.3mm. Electrical conductivity was achieved by inserting a silver wire (0.5 mm in diameter) into the diamond paste. Before each use, the electrode surface was smoothed by polishing with alumina foils (polishing strips 30144-001, Orion). All sensors were stored at room temperature, when not in use.

6.2.3 Recommended procedures

6.2.3.1 Cyclic voltammetry

For the optimization step, all cyclic voltammetry scans between –1.0 and +1.0 V were carried out. The diamond paste-based sensors were used as working electrodes, an Ag/AgCl was used as reference electrode and a Pt wire as counter electrode. The scan

rate was adjusted to 20mV/s. The experiments were carried out under deaerated conditions.

6.2.3.2 Direct differential pulse voltammetry (DPV)

The technique used for the direct assay was differential pulse voltammetry with applied potential pulse amplitude of 25 V, scan rate of 20 mV/s, pulse width of 50 ms, and pulse time of 40 ms. All measurements were carried out at 25 ^{o}C. The diamond electrode together with the reference and auxiliary electrodes were dipped into the compartment cell containing 10^{-4} mol/L of L- or D-Fucose, phosphate buffer and supporting electrolyte (0.1mol/L). All conditions of different DPV scan measurements are summarized in Table 6.1. The peak heights of different electrodes, measured at the suitable potentials, were plotted versus the concentrations of L- or D-fucose. The unknown concentrations of L- and D-fucose were determined from the calibration graphs.

6.2.4 Reagents and materials

All solutions were prepared by using deionized water (A Modulab System, Continental Water Systems, San Antonio, TX). L- and D-fucose, monocrystalline natural diamond powder (1 μm) (99.9%) and monocrystalline synthetic diamond powder 1 μm (synthetic-1) (99.9%) and 50 μm (synthetic-2) (99.9%) powder were purchased from Aldrich (Milwaukee, WI). The paraffin oil was purchased from Fluka (Buchs, Switzerland). 250 mL of 10^{-2} mol/L of L- and D-fucose were prepared as stock solutions. All solutions of different concentrations were prepared from the stock solutions by serial dilutions. Phosphate buffer (pH 7) was prepared from KH_2PO_4 (Saarchem-Holpro Analytic)) and

Na_2HPO_4 (Chemical Suppliers) using de-ionized water. Solutions of different pH values (1-10) were prepared by the addition of HCl or NaOH solutions (0.1mol/L) to the solutions containing L- or D-fucose. The pH value was monitored using pH-meter (Orion, Model 420A, Labotec).

6.2.5 L-fucose samples.

Three serum samples (1-3) and three urine samples (4-6) of suspected cancer patients were provided from local hospitals. The direct DPV method was used for the analysis of L-fucose concentrations in the serum and urine samples.

6.3 Results and discussion

6.3.1 Optimization of working conditions

Optimization of working conditions was carried out for the different types of diamond paste-based electrodes using L- and D-fucose as analytes. Cyclic voltammograms for different electrolyte solutions and pHs (1-10) were recorded before and after the addition of different aliquots of L- or D-fucose, separately. Four different supporting electrolytes (0.1mol/L NaCl, 0.1mol/L KCl, 0.1mol/L $NaNO_3$, and 0.1mol/L KNO_3) were tested. Figures 6.2 and 6.3 show the effect of pH and supporting electrolyte on the peak height for L-fucose and D-fucose, respectively. The shapes and heights of the peaks contribute to the selection of the optimum electrolyte and pH. The best peak shapes for the three L-fucose assay electrodes were recorded at: pH 3.0 using NaCl as electrolyte, at pH 10.0 using KNO_3 as electrolyte, and at pH 10.0 using KCl as electrolyte for natural diamond, synthetic 1 and synthetic 2 base electrodes.

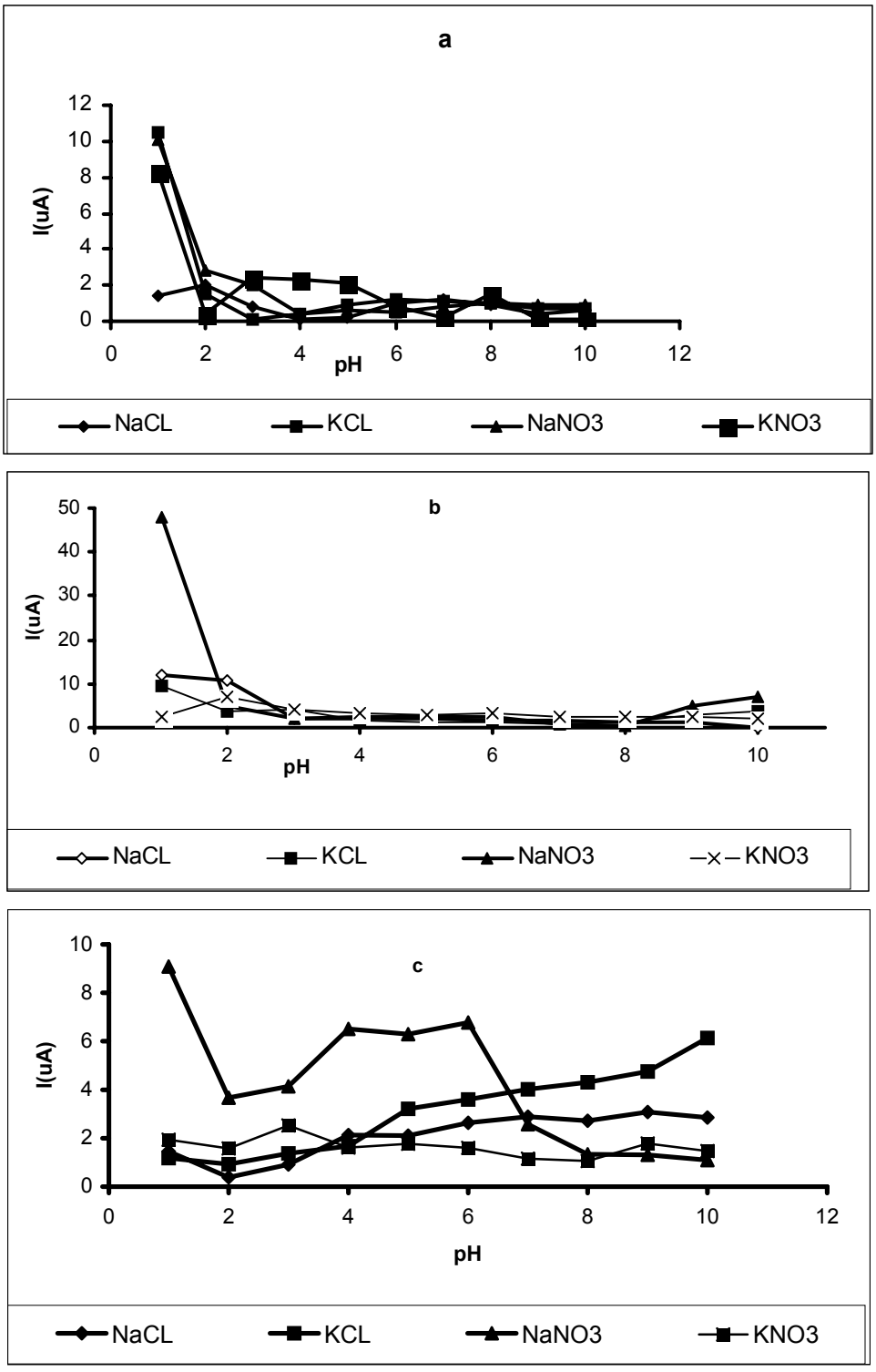

Figure 6.2 The effect of electrolyte and pH on the peak height (μA) for a concentration of L-Fucose solution of 10^{-4} mol/L when (a) natural diamond, (b) synthetic-1, and (c) synthetic-2 based electrodes are used.

The peaks measured a pH 1.0 in 0.1 mol/L of $NaNO_3$ were approximately higher than the peaks recorded for the rest of the electrolytes at different pHs. Despite of these highest peaks, they were broad causing poor resolution which enhances the effect of interferences in the determination of L-fucose in serum and urine samples. As well, the optimum conditions for D-fucose electrodes based on natural diamond, synthetic-1, and synthetic-2 diamonds were recorded at: pH 1.0 using KCl as electrolyte, at pH 1.0 using KNO_3 as electrolyte, and at pH 1.0 using $NaNO_3$ as electrolyte. Table 6.1 summarizes the optimum working conditions, pH and supporting electrolyte, for L- and D-fucose assay using the diamond paste based electrode.

Table 6.1 Optimum conditions for L- and D-fucose determination

Analyte	Optimization factor	**Electrodes Based on**		
		Natural diamond	Syntheic-1	Synthetic-2
L-fucose	Electrolyte (0.1mol/l)	NaCl	KNO_3	KCl
	pH	3.00	10.00	10.00
	Potential range (mV)	0.00-500	0.00-500	0.00-300
	Peak Position(mV)	240	130	90
	Background current (μA)	0.895	0.5967	0.068
D-fucose	Electrolyte (0.1mol/l)	KCl	KNO_3	$NaNO_3$
	pH	1.00	1.00	3.00
	Potential range (mV)	0.00-350	0.00-350	-50.00-350
	Peak Position(mV)	120	130	130
	Background current (μA)	17.43	3.722	0.232

All measurements were made at 25^{o}C; all values are the average of ten determinations.

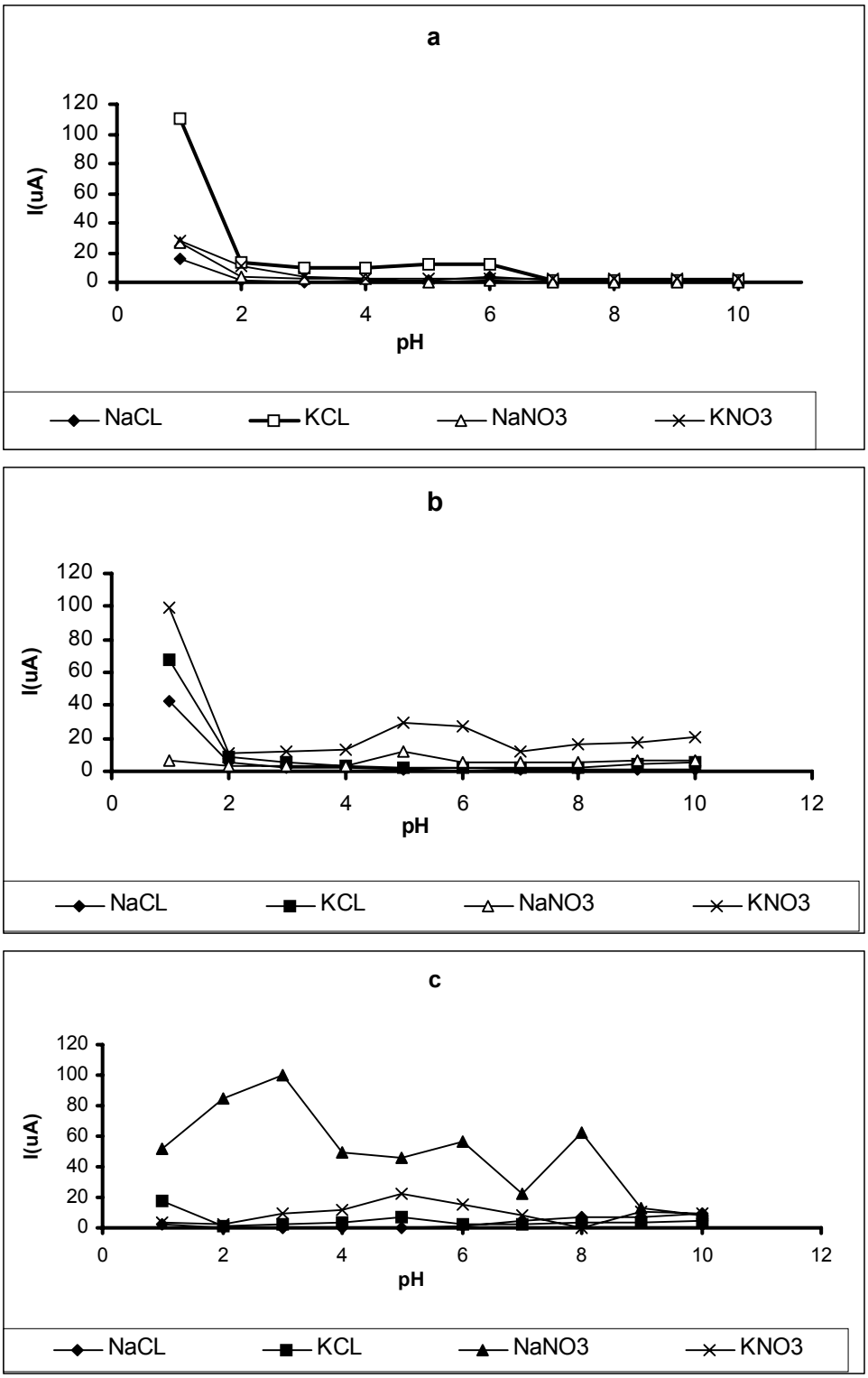

Figure 6.3 The effect of electrolyte and pH on the peak height (μA) for a concentration of D-Fucose solution of 10^{-4} mol/L when (a) natural diamond, (b) synthetic-1, and (c) synthetic-2 based electrodes are used..

6.3.2 Response of the diamond paste based electrodes

The response characteristics of the diamond paste-based electrodes for L- and D-fucose were determined using DPV. The responses of the electrodes were linear over wide concentration ranges and can be described by the calibration equations for L- and D-fucose (Table 6.2). The limits of detections were calculated using the following equation [31]:

$$DL = \frac{I_B + 3\sigma_S - a}{S} \qquad \textbf{(6.1)}$$

where I_B is the background current recorded, σ_S is the standard deviation for the measurement of the background current, a is the slope of the calibration equation.

Table 6.2 Response characteristics of the diamond paste-based amperometric electrodes for L- and D-fucose assay**.**

		Electrodes based on		
		Natural diamond	Synthetic-1	Synthetic-2
L-fucose	Equation of calibration	$H^a=1.38+1.99C^d$	$H^a=1.13+3.35C^e$	$H^b=10.67+32.04C^c$
	Linear concentration range (mol/L)	$10^{-13} – 10^{-9}$	$10^{-11} – 10^{-8}$	$10^{-6} – 10^{-3}$
	Limit of detection (mol/L)	10^{-14}	10^{-12}	10^{-8}
	Regression coefficient (r)	0.9994	0.9940	0.9931
D-fucose	Equation of calibration	$H^a=0.48+0.2C^c$	$H^a=1.41+0.08C^c$	$H^a=3.74+3.66C^c$
	Linear concentration range (mol/L)	$10^{-6} – 10^{-3}$	$10^{-5} – 10^{-3}$	$10^{-9} – 10^{-3}$
	Limit of detection (mol/L)	10^{-7}	10^{-7}	10^{-10}
	Regression coefficient (r)	0.9999	0.9994	0.9917

[a] All measurements were made at 25°C; all values are the average of ten determinations H is the peak height ([a] μA and [b] nA), C is the concentration of L- or D-fucose ([c] mmol/L, [d] nmol/L, and [e] pmol/L) and r is the regression coefficient.

The peak heights for natural diamond, synthetic-1, and synthetic-2 diamonds based electrodes were proportional to the L- and D-fucose concentrations with low detection limits as shown in table 6.2. A previous comparison analysis using monocrystalline diamonds (natural, synthetic-1, and synthetic-2), glassy carbon (GC), and carbon paste (CP) electrodes was shown by our group [32]. The signal to noise ratio for the diamond paste-based electrodes was higher comparing to GC and CP [32]. The diamond paste-based electrodes proved a good reproducibility of peak current according to the obtained values of standard deviations (RSD < 1%).

6.3.3 Selectivity of the diamond paste electrodes

The selectivity of all the diamond paste-based electrodes was checked. Mixed and separate solutions method was used to calculate the amperometric selectivity coefficients [30]. The ratio between L- or D-fucose and the interfering species was 1:10 (mol/mol). The amperometric selectivity coefficients for diamond paste-based electrodes (Table 6.3) proved the enantioselectivity of the proposed electrodes. Accordingly, these results proved that L- and D-fucose can be analyzed one in the presence of the other, using the proposed electrodes.

Table 6.3 Amperometric Selectivity Coefficients[a]

Analyte(I)/ Interfering species (J)	K_{sel}^{amp}		
	Electrodes based on		
	Natural diamond	Synthetic-1	Synthetic-2
L-fucose/D-fucose	3.95×10^{-3}	7.05×10^{-3}	4.19×10^{-4}
D-fucose/L-fucose	4.06×10^{-3}	4.41×10^{-3}	1.21×10^{-3}

[a] All measurements were made at 25°C; all values are the average of ten determinations.

6.3.4 Analytical applications

The optimum working conditions (Table 6.1) were used for all recovery tests. The suitability of the proposed diamond paste electrodes for the enantioanalysis of fucose was determined using solutions containing both enantiomers in different ratios. The recovery values for the assay of one enantiomer in the presence of its antipode proved no significant differences in these results for the ratios between L:D or D:L enantiomers varying from 2:1 to 1:9 (RSD <1%), (Tables 6.4 and 6.5).

Table 6.4 Recovery of L-fucose in the presence of D-fucose

	Average recovery, (%)[a]		
L:D (mol:mol)	Electrodes based on Natural diamond	Electrodes based on Synthetic-1	Electrodes based on Synthetic-2
2:1	99.92±0.03	99.97±0.03	99.95±0.04
1:1	99.90±0.02	99.99±0.04	99.93±0.03
1:2	99.89±0.02	99.96±0.04	99.94±0.03
1:4	99.96±0.03	99.97±0.04	99.95±0.04
1:9	99.92±0.03	99.95±0.04	99.96±0.03

[a] All measurements were made at 25°C; all values are the average of ten determinations.

Table 6.5 Recovery of D-fucose in the presence of L-fucose

	Average recovery, (%)[a]		
D:L (mol:mol)	Electrodes based on Natural diamond	Electrodes based on Synthetic-1	Electrodes based on Synthetic-2
2:1	99.48±0.03	99.62±0.02	99.56±0.03
1:1	99.67±0.03	99.76±0.03	99.63±0.03
1:2	99.99±0.02	99.86±0.03	99.76±0.02
1:4	99.98±0.03	99.85±0.02	99.70±0.02
1:9	99.99±0.02	99.87±0.02	99.69±0.03

[a] All measurements were made at 25°C; all values are the average of ten determinations.

The results obtained demonstrated the suitability of diamond paste electrodes for the enantioanalysis of fucose in biological fluids (serum and urine) (Table 6.6). The recovery tests performed for the assay of L-fucose in serum and urine samples using DPV were higher than 99.00% with RSD < 1% (n=10). Due to precision and accuracy of results, diamond electrodes can be reliably used for the enantioselective assay of L-fucose in serum and urine samples with a high average recovery and low RSD % values.

Table 6.6 Recovery of L-fucose from serum and urine samples using electrodes based on natural diamond, synthetic 1 and synthetic 2

Type of sample	L-fucose, Average recovery		
	Sample No.	Standard method [27]	Amperometric electrodes
Electrodes based on Natural diamond (pmol/L)			
Serum	1	60	59.73±0.024
	2	233	232.25±0.07
	3	333	331.83±0.1
Urine	4	430	428.67±0.129
	5	500	498.40±0.20
	6	910	906.18±.0.273
Electrodes based on Synthetic-1 (nmol/L)			
Serum	1	6.00	5.99±0.01
	2	23.3	23.24±.0.01
	3	33.3	33.23±0.01
Urine	4	43.0	42.90±0.02
	5	50.0	49.87±0.02
	6	91.0	90.76±0.02
Electrodes based on Synthetic-2 (μmol/L)			
Serum	1	18.0	17.92±0.01
	2	70.0	69.75±0.02
	3	100.0	99.69±0.04
Urine	4	280.0	279.10±0.11
	5	500.0	498.45±0.15
	6	910.0	907.82±0.36

[a] All measurements were made at 25°C; all values are the average of ten determinations

6.3.5 Statistical comparison between diamond paste electrodes and the standard method for fucose analysis

Statistical comparison between the results obtained for L- and D-fucose using the diamond paste electrodes and the standard method were performed for all serum and urine samples using the paired t-test. These data were compared statistically with the standard method at the 99.00% confidence level. The two methods are compared by analyzing several different samples. The comparison refers to paired data, and t_{calc} is computed by the expression [33],

$$t_{calc} = \left| \bar{X}_A - \bar{X}_B \right| \sqrt{\frac{N(N-1)}{\sum (d_i - \bar{d})^2}} \qquad (6.2)$$

where $\bar{X}_A$ and $\bar{X}_B$ are the average recoveries of L-fucose using standard method and proposed diamond paste electrodes method, respectively. N is number of paired samples, which are analyzed by both methods, d_i is the individual difference between the two methods for each sample, and $\bar{d}$ is the mean of all d_i values. t_{calc} values were 2.96, 0.89, and 2.37 for natural, synthetic-1, and synthetic-2 diamond paste electrodes method versus the standard method. All t_{calc} values, at the 99.00% confidence level, are less than the tabulated theoretical t-value: 4.032. Since all experimental t_{calc} are less than theoretical t-value, there is no statistically significant difference between the results obtained by the standard method and diamond paste electrodes.

6.4 Conclusion

Monocrystalline diamonds (natural, synthetic-1, and synthetic-2) were found to be excellent materials for the electrodes designs, and enantioanalysis of L- and D-fucose. The electrodes were enantioselective, precise, and accurate with low detection limits. The design is simple and the electrodes exhibit a long term stability. The low RSD (<1%) values of the recovery tests using DPV, proved the reliability of analytical results. This work is opening an excellent vision of miniaturization of the electrodes for application for *in vivo* analysis of L-fucose in patients with various types of cancer.

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