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Carbon Paste and Modified Carbon Nanotubes Paste Sensors for Determination of Reducing-Osteoarthritis Drug Glucosamine Sulphate in Bulk Powder and in its Pharmaceutical Formulations

Nawal A. Al-arfaj, Maha F. El-Tohamy[*]

Department of Chemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia
[*]E-mail: star2000star@gmail.com

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In the present study newly developed potentiometric sensors for determination of glucosamine sulphate are presented. The proposed method based on the fabrication of two sensors carbon paste (CP) and modified carbon nanotubes carbon paste (MCNTs) sensors. The developed sensors were fabricated by the incorporation of glucosamine sulphate with sodium tetraphenyl borate (TPB) forming glucosamine-tetraphenyl borate as electroactive material. The proposed sensors showed Nernstain response (57.03±0.4 and 58.24±0.7 mV decade^{-1}) over linear concentration range 1.0x10^{-6}-1.0x10^{-2} and 5.0x10^{-7}-1.0x10^{-1} mol L^{-1} for CP and MCNTs sensors, respectively. The lower limit of detection for both sensors was investigated and they recorded a valid response at 5.0x10^{-7} and 2.5x10^{-7} mol L^{-1}. The performance characteristics for the proposed sensors were optimized and evaluated. The influence of common and possible foreign substances on the response of the sensors was tested using separate solution method. The obtained results showed no interference. The results were statistically validated and the proposed method has been successfully applied for determination of glucosamine in its bulk powder and pharmaceutical formulations.

Keywords: Modified carbon nanotubes carbon paste sensor, potentiometric analysis, glucosamine sulphate, pharmaceutical formulations, Osteoarthritis

1. INTRODUCTION

Glucosamine sulphate (Figure 1) is chemically known as Bis (2-ammonio-2-deoxy-D-glucose) sulphate. It is a naturally occurring chemical found in the human body. It exists in the fluid that is around joints. Glucosamine sulphate is commonly used for arthritis. Scientists have studied it

extensively for this use. It is most often used for a type of arthritis called osteoarthritis, which is the most common type of arthritis. Glucosamine sulphate is also used in some skin creams to control arthritis pain. These creams usually contain camphor and other ingredients in addition to glucosamine [1].

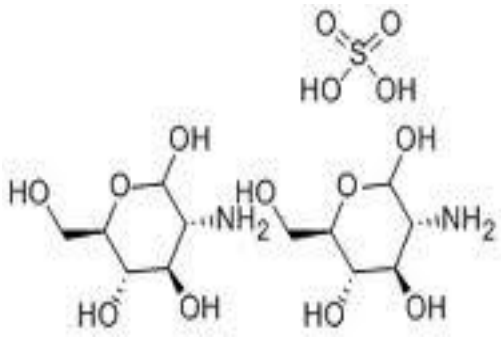

Figure 1. Chemical structure of glucosamine sulphate

The literature survey shows that there are several methods for determination of glucosamine including high- performance liquid chromatography [2-4], Liquid chromatography coupled with mass spectrometry [5-7], capillary zone electrophoresis [8, 9], thin layer chromatography [10] and spectrophotometry [11].

From the previous literature survey, no electrochemical sensors have been developed for the determination of glucosamine sulphate yet. The goal of choice for the determination of the investigated drug using carbon paste sensor and modified nano carbon paste was attributed to the simplicity of the fabrication of sensors, less time consuming, low cost and the proposed sensors can be used without pre-treatment of samples. Also compared with other previous chromatographic techniques the use of modified nano carbon paste sensor exhibited a good stability and reproducibility.

In the present paper the aim of the work is the fabrication, optimization and characterization of new validated sensors for the determination of glucosamine sulphate in bulk powder and in pharmaceutical formulations.

2. EXPERIMENTAL

2.1. Materials and reagents

All chemicals and reagents used were of analytical grade. Pure grade glucosamine sulphate was kindly supplied from Adwia Co. Egypt. High purity graphite powder (1-2 μm) and multi-wall carbon nanotubes powder (carbon >95.0%, O.D. x L 6-9 nm x 5 μm) were purchased from Sigma-Aldrich, Germany. Sodium tetraphenyl borate >99.5 %, dioctylphthalate (DOP) 99.5%, di-butyl phthalate (DBP) 99.0%, di-butyl sebacate (DBS) ≥ 97.0%, di-octyl sebacate (DOS) ≥ 97.0%, nitrophenyloctylether (*o*-NPOE) 99.0% and tri-butyl phosphate (TBP) 98.0% were provided by Fluka,

Switzerland. The pharmaceutical preparation (Just vitamins® 1500 mg/tablet) was purchased from local drug stores.

2.2. Apparatus

HANNA instruments pH 211 microprocessor pH-meter was used for all experimental measurements. Saturated calomel electrode (SCE) was used as an external reference electrode while Ag/AgCl electrode was used as an internal reference electrode.

2.3. Standard drug solution

A Stock glucosamine sulphate solution 0.1 mol L^{-1} was freshly prepared daily by dissolving 1.1410 g in 25 mL distilled water. Working solutions ($1.0x10^{-8}$-$1.0x10^{-1}$ mol L^{-1}) were prepared by appropriate dilution with distilled water.

2.4. Preparation of glucosamine-tetraphenyl borate ion pair

The ion-pair was prepared by mixing 50 mL of equimolar $1.0x10^{-2}$ mol L^{-1} for both glucosamine and sodium tetraphenyl borate. The resulting white precipitate was filtered, washed thoroughly with distilled water and air dried at room temperature for 24 h.

2.5. Sensor construction

Carbon paste sensor: The homogenous carbon paste sensor was prepared by hand mixing of 60.0% pure graphite powder (1-2 μm) with 30.0 % *o*-NPOE as plasticizing liquid and 10.0% ion-pair (glucosamine-tetraphenyl borate) in an agate mortar. Then the carbon paste was carefully packed in Teflon tube 3 mm in diameter). A shiny, smooth and fresh surface was achieved by polishing the carbon paste surface using transparent paper.

Modified multi-wall carbon nanotubes carbon paste sensor: The modified multi-wall carbon nanotubes carbon paste sensor was prepared by the same steps as previously mentioned for carbon paste sensor but in the modified sensor a small amount of carbon nano particles was added and the paste was homogenously mixed. Then the packed sensor was dried in air for 24 h.

2.6. Sensor calibration

In order to calibrate the fabricated sensors all potentiometric measurements were recorded using the proposed sensor(s) in conjunction with double junction Ag/AgCl reference electrode. The calibration graphs were carried out using 100 mL of standard drug solutions $1.0x10^{-8}$-$1.0x10^{-1}$ mol L^{-1}. The measured potential was plotted against the logarithm of glucosamine sulphate drug concentration.

2.7. Sensor selectivity

The influence of common and possible foreign substances such as different cationic species, sugars, amino acids and some additive formulated substances on the selectivity of the fabricated glucosamine sensors were investigated using separate solution method [12]. The calculated selectivity coefficients of the proposed sensors were carried out using the following equation:

$$\text{Log } K^{Pot}_{GLuco^-, J^{z+}} = (E_2 - E_1)/S + \log [\text{Gluco.}] - \log [J^{z+}]^{1/z}$$

Where, E_1 is the electrode potential in $1.0\text{x}10^{-3}$ mol L^{-1} glucosamine sulphate solution. E_2 is the potential of the electrode in $1.0\text{x}10^{-3}$ mol L^{-1} solution of the interferent ion J^{z+} and S is the slope of the calibration plot.

2.8. Effect of pH

The effect of pH on the fabricated sensor(s) potential was investigated. The pH of glucosamine sulphate $1.0\text{x}10^{-4}$ mol L^{-1} was gradually increased or decreased using small volumes of $1.0\text{x}10^{-1}$ mol L^{-1} of sodium hydroxide of hydrochloric acid. The recorded potential response was plotted as a function of pH using pH/mV meter.

2.9. Effect of plasticizers

The influence of different types of plasticizers on the performance characteristics of the fabricated sensors was tested. The calibration parameters were recorded using six types of plasticizers di-octyl phthalate (DOP), di-butyl sebacate (DBS), di-octyl sebacate (DOS), di-butyl phthalate (DBP), tri-butyl phosphate (TBP) and *o*-nitrophenyloctyl ether (*o*-NPOE). The quality of the proposed sensors was evaluated in relation to life-time, slope, detection limit and response time.

2.10 Analytical Applications

2.10.1. Determination of glucosamine sulphate in tablets

Five tablets of Just vitamins® (1500 mg glucosamine sulphate/tablet) were finally powdered and mixed. An accurate amount of the powdered drug equivalent to 100 mg of glucosamine sulphate was dissolved in 100 mL distilled water. The working solutions of the investigated drug were prepared in the range of $1.0\text{x}10^{-6}$-$1.0\text{x}10^{-2}$ mol L^{-1} by appreciate dilution using distilled water.

Standard addition method was used for the determination of the investigated drug in its dosage form by using both glucosamine-tetraphenyl borate CP sensor and MCNTs sensor.

2.10.2. Content uniformity assay of glucosamine sulphate tablets

Ten individual tablets of glucosamine sulphate 1500 mg/ tablet were dissolved in 100 mL of distilled water. The fabricated sensors were used for the determination of the content uniformity assay of the investigated tablets. Each sensor was immersed in the drug sample separately. The mean potential was recorded and used to evaluate the content uniformity from the calibration graph.

3. RESULTS AND DISCUSSION

3.1. Nature and response characteristics of the sensors

Glucosamine sulphate reacts with tetraphenyl borate to form electroactive material glucosamine-tetraphenyl borate which found to be water insoluble but readily in an organic solvent such as tetrahydrofuran (THF). The proposed sensors were optimized and characterized for the determination of glucosamine sulphate. The nature and response characteristics of glucosamine CP and MCNTs sensors were investigated and the obtained results were summarized in Table 1.

Table 1. Critical response characteristics of glucosamine-tetraphenyl borate carbon paste and modified carbon nanotubes paste sensors

Parameter[a]	Glucosamine-TPB carbon paste sensor	Glucosamine-TPB modified carbon nanotubes sensor
Slope (mV decade^{-1})	57.03±0.4	58.24±0.7
Intercept	466.05	531.73
Correlation coefficient r	0.9997	0.9998
Linear range (mol L^{-1})	1.0x10^{-6}-1.0x10^{-2}	5.0x10^{-7}-1.0x10^{-1}
LOD (mol L^{-1})	5.0 x10^{-7}	2.5 x10^{-7}
Response time (s)	30	≥ 15
Working pH range	3-9	3-9
Lifetime /day	30	45
Accuracy (%)	99.36	99.72
Standard deviation	0.3	0.2
Robustness[b]	98.99±0.68	99.63±0.37
Ruggedness[c]	99.41±0.42	99.67±0.33

[a]Mean of six measurements [b]A small variation in method parameters were carried out as pH of phosphate buffer (pH 8±1). [c] Comparing the results by those obtained by different sensors assemblies using (Jenway 3510 pH meter)

The proposed sensors showed Nernstain response with slopes 57.03±0.4 and 58.24±0.7 mV decade^{-1} over concentration ranges from 1.0x10^{-6}-1.0x10^{-2} and 5.0x10^{-7}-1.0x10^{-1} mol L^{-1} with limits of detection 5.0x10^{-7}and 2.5x10^{-7} mol L^{-1} for CP and MCNTs sensors, respectively (Figure 2). The

results showed that the modified sensor using carbon nano particles exhibits the best performance characteristics than that fabricated from carbon paste.

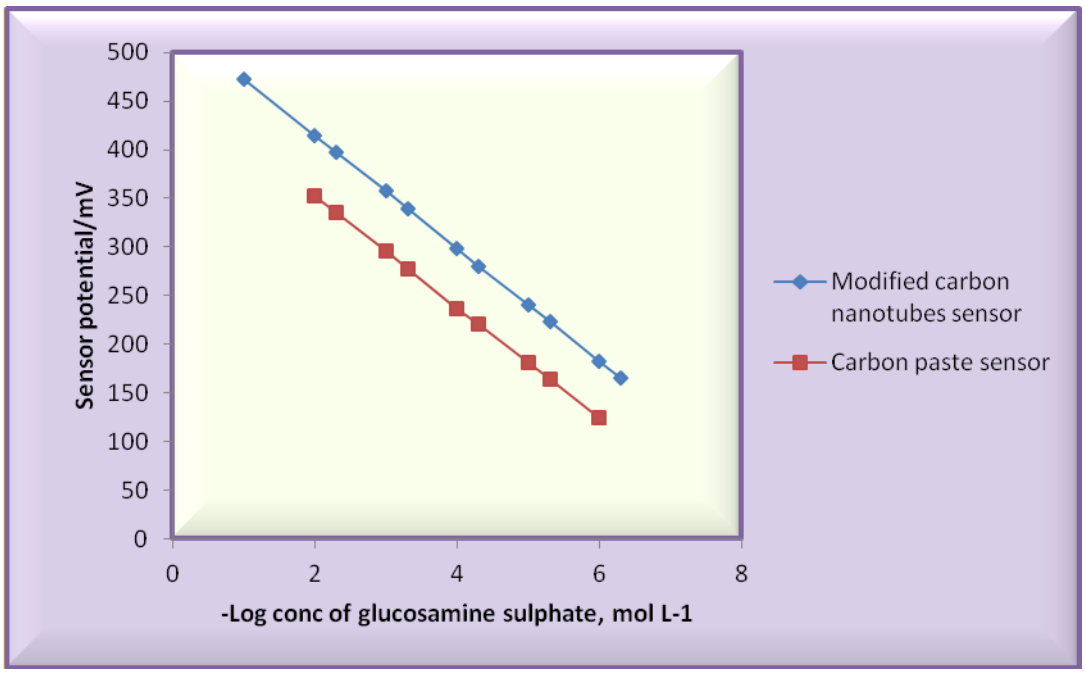

Figure 2. Typical calibration graphs for glucosamine sensors

The life time of the fabricated sensors was examined using drug concentration ranging from $1.0x10^{-8}$-$1.0x10^{-1}$ mol L^{-1} and the sequence of measurements was from low to high concentrations. It has been found that both sensors exhibited a dynamic response 30 and ≥15 s for a period of 30 and 45 days for CP and MCNTs sensors, respectively. The obtained results revealed that the use of modified carbon nanotubes carbon paste sensor exhibit better concentration range, response time and sensor stability.

3.2. Effect of plasticizer

In the present study, five plasticizers (DOS), (o-NPOE), (DBP), (DOP) and (TBP) were used to optimize the performance of the prepared sensors. On the basis of the calibration parameters, some properties of sensors in relation with their slope, life time, linear concentration ranges and lower limit of detections were investigated using calibration graphs. The obtained results (Table 2) revealed that the most sensible values of slope (56.05±0.5 and 57.96±0.9 mV $decade^{-1}$) for CP and MCNTs, respectively were corresponded to sensors fabricated from (*o*-NPOE). The use of *o*-NPOE as solvent mediator showed a lower limit of linear response values $5.0x10^{-7}$ and $2.5x10^{-7}$ for the previously mentioned sensors, respectively. The lipophilicity of the solvent mediator avoids the leaching of the mediator from the surface of the sensor in the test solution [13].

Table 2. Calibration parameters by employing different kinds of plasticizers for glucosamine-TPB sensors

Type of sensors	Parameters	DOS	*o*-NPOE	DBP	DOP	TBP
CP	Slope (mV decade^{-1})	52.60±0.2	56.50±0.5	55.54±0.9	53.89±0.4	51.97±0.1
	Correlation coefficient, r	0.9987	0.9997	0.9994	0.9988	0.9978
	Linear Conc. range mol L^{-1}	1.0x10^{-5}-1.0x10^{-2}	1.0x10^{-6}-1.0x10^{-2}	5.0x10^{-6}-1.0x10^{-3}	1.0x10^{-5}-1.0x10^{-3} 4.9x10^{-6}	1.0x10^{-5}-5.0x10^{-3} 4.5x10^{-6}
	LOD	5.0x10^{-6}	5.0x10^{-7}	2.5x10^{-6}	20	15
	Life time/days	25	30	30		
MCNTs	Slope (mV decade^{-1})	54.20±0.6	57.96±0.9	55.85±0.3	52.36±0.8	51.18±0.6
	Correlation coefficient, r	0.9989	0.9999	0.9998	0.9988	0.9987
	Linear Conc. range mol L^{-1}	1.0x10^{-6}-1.0x10^{-2}	5.0x10^{-7}1.0x10^{-1} 2.5x10^{-7}	1.0x10^{-6}-1.0x10^{-2}	1.0x10^{-5}-1.0x10^{-2} 5.0x10^{-6}	1.0x10^{-5}-1.0x10^{-2} 4.6x10^{-6}
	LOD	4.6x10^{-7}	45	3.2x10^{-7}	30	18
	Life time/days	20		30		

Also, it can be seen that the values obtained for correlation coefficients (0.9994 and 0.9999) for the previously mentioned sensors evidenced the good linearity of calibration curves.

The lipophilicity of the proposed sensors fabricated by *o*-NPOE plays an important role in the lower loss of the electroactive materials during the experimental analysis and therefore gave longer lifetime than those with lower lipophilicity.

Moreover, the relation of the dielectric constant (ε_r) of plasticizers DOS (ε_r = 3.9), *o*-NPOE (ε_r = 23.9), DOP (ε_r = 5.1), DBP (ε_r = 6.4) and TBP (ε_r =8.0) against the life time of the fabricated sensors was investigated and plotted (Figure 3) and from the results obtained it has been seen that the most preferable plasticizer was *o*-NPOE with ε_r=23.9.

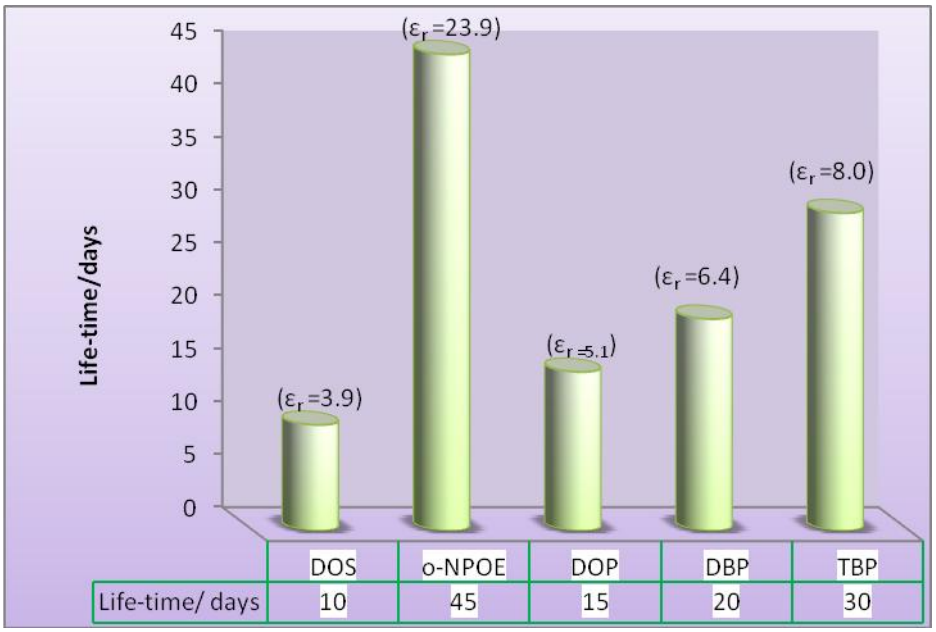

Figure 3. Life-time of the glucosamine-tetraphenyl borate sensors as function of dielectric constant (ε_r) of plasticizers

3.3. Effect of pH

The pH dependence of the investigated sensors was examined using glucosamine sulphate solution $1.0x10^{-3}$ mol L^{-1}. The pH value was gradually increased and decreased using 0.1 mol L^{-1} sodium hydroxide and hydrochloric acid, respectively. The potential of the sensors was recorded and plotted against –log glucosamine sulphate solutions. Below pH 3, with the increase of the analyte acidity the potential was increased which may be due to the extraction of H^+ ions by membrane. While at more than 9 the potential response was decreased, this may be attributed to the decrease of analyte ion by the increase of OH- concentration [14].

The obtained results were shown in Figure 4 indicating the safe pH range at 3-9.

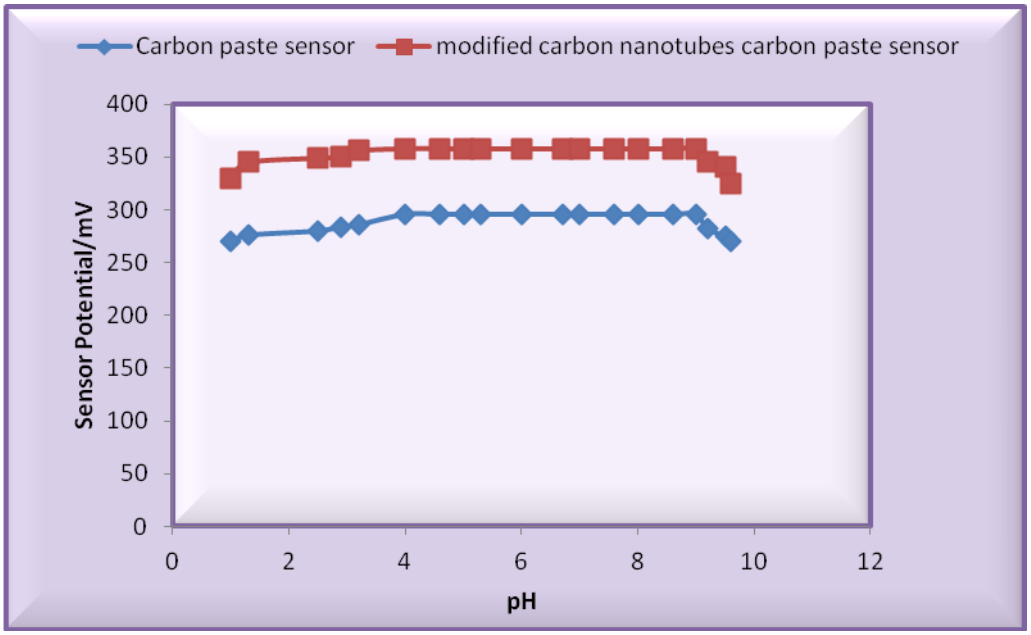

Figure 4. Effect of pH on glucosamine-tetraphenyl borate sensors

3.4. Selectivity of the sensors

The potentiometric selectivity coefficients of the prepared sensors were investigated using separate solution method [12] using inorganic cations such as $Na^{+,}$ K^+, NH_4^+, Ca^{2+}, Mg^{2+} , Zn^{2+}, Al^{3+} and Fe^{3+}. The obtained results in Table 3 showed high selectivity of the sensors in the presence of such cations. Also the selectivity of the sensors was tested in the presence of some sugars, additive compounds such as magnesium stearate, stearic acid and amino acids. The calculated results revealed that there is no interference and no need to avoid these compounds during the application of the proposed sensors. Moreover, using the modified carbon nanotubes carbon paste sensors exhibited high selectivity and reproducibility for the determination of glucosamine sulphate.

Table 3. Selectivity coefficients for glucosamine-TPB sensors using separate solution method ($1.0x10^{-3}$ mol L^{-1} glucosamine sulphate)

Interferent	$K^{Pot}_{Glucos.}$	
	Glucosamine-TPB CP sensor	Glucosamine-TPB MCNTs sensor
Na^+	$1.6x10^{-3}$	$2.1x10^{-4}$
K^+	$3.1x10^{-3}$	$4.8x10^{-4}$
NH_4^+	$4.3x10^{-3}$	$2.3x10^{-3}$
Ca^{2+}	$2.1x10^{-3}$	$4.1x10^{-4}$
Mg^{2+}	$7.8x10^{-3}$	$1.1x10^{-3}$
Zn^{2+}	$1.6x10^{-3}$	$2.0x10^{-3}$
Cu^{2+}	$1.4x10^{-3}$	$1.8x10^{-4}$
Fe^{3+}	$2.2x10^{-3}$	$6.4x10^{-4}$
Al^{3+}	$8.5x10^{-4}$	$4.3x10^{-4}$
Glucose	$6.4x10^{-4}$	$6.9x10^{-4}$
Lactose	$7.3x10^{-4}$	$8.7x10^{-4}$
Histadine	$4.8x10^{-4}$	$3.6x10^{-4}$
L- Leucine	$1.2x10^{-3}$	$2.9x10^{-4}$
L-Valine	$8.5x10^{-3}$	$4.7x10^{-4}$
L-Cystine	$5.9x10^{-3}$	$2.6x10^{-4}$
Glycine	$8.9x10^{-4}$	$1.8x10^{-4}$
Starch	$5.7x10^{-4}$	$5.3x10^{-4}$
Magnesium stearate	$3.2x10^{-4}$	$3.1x10^{-4}$

3.5. Quantification of glucosamine sulphate

Table 4. Statistical treatment of the data obtained for the determination of glucosamine sulphate in pure form by the proposed and reported method [11]

Type of sensor	Taken mol L^{-1}	Mean recovery %	n	Variance	SD	%SE**	% RSD	t-test	F-test
Glucosamine–TPB CP sensor	$1.0x10^{-6}$-$1.0x10^{-2}$	99.47	7	0.201	0.448	0.169	0.450	0.987 (2.201)*	2.03(4.39)*
Glucosamine-TPB MCNTs sensor	$5.0x10^{-7}$-$1.0x10^{-1}$	99.59	8	0.529	0.727	0.187	0.729	0.396 (2.179)*	2.63(3.97)*
Reported method	$1.0x10^{-6}$-$1.0x10^{-2}$	99.68	6	0.099	0.315	0.129	0.316		

*The Figures in parentheses are the tabulated t- and F- test at p = 0.05[15] ** %Error= %RSD/√n

Direct potentiometric determination for glucosamine sulphate in pure form using the fabricated sensors was carried out. The mean percentage recoveries were 99.47±0.44 and 99.59±0.73 for carbon paste and modified carbon nanotubes carbon paste, respectively. Furthermore, the results obtained were encouraging so the proposed method was applied for the determination of glucosamine sulphate

in its pharmaceutical preparations. The results were compared with the reported spectrophotometric method [11] and the results are listed in Table 4 and 5.

Table 5. Statistical treatment of the data obtained for the determination of glucosamine sulphate in Just vitamins® tablets form by the proposed and reported method [11]

Type of sensor	Taken mol L^{-1}	Mean recovery %	n	Variance	SD	%SE**	% RSD	t-test	F-test
Glucosamine–TPB CP sensor	$1.0x10^{-6}$-$1.0x10^{-2}$	99.58	7	0.195	0.442	0.167	0.444	0.519(2.201)*	3.54(4.39)*
Glucosamine-TPB MCNTs sensor	$5.0x10^{-7}$-$1.0x10^{-1}$	99.56	8	0.169	0.412	0.146	0.414	0.687(2.179)*	3.07(3.97)*
Reported method	$1.0x10^{-6}$-$1.0x10^{-2}$	99.68	6	0.055	0.235	0.096	0.236		

3.6. Content uniformity assay of glucosamine sulphate tablets

The proposed glucosamine-tetraphenyl borate sensors were used for the determination of content uniformity assay of glucosamine sulphate® in tablets (1500 mg /tablet). The content of tablets was calculated from the regression equations for the proposed sensors. The results obtained as mean% recoveries and standard deviations were 98.79±0.99 and 99.19±0.56 for carbon paste and modified carbon nanotubes carbon paste sensors, respectively. The proposed sensors showed good accuracy and high precision for routine quality control analysis.

3.7. Method validation

The method was validated for linearity, accuracy, precision, repeatability, robustness and ruggedness accordance with ICH guidelines [16].

3.7.1. Linearity and lower limit of detection

Under optimal experimental conditions the linearity of the proposed method was investigated by plotting the potential of the fabricated sensors/mV as a function of logarithm corresponding concentration of the tested drug. It has been shown that the fabricated sensors exhibit Nernstian response over concentration ranges of 1.0×10^{-6}-1.0×10^{-2} mol L^{-1} and 5.0×10^{-7}-1.0×10^{-1} with lower limits of detection of $5.0x10^{-7}$ and $2.5x10^{-7}$ mol L^{-1} for CP and MCNTs sensors, respectively. It is obvious that the use of modified multi-wall nano carbontubes carbon paste improve the sensitivity for detection of very small concentration of glucosamine sulphate.

3.7.3. Robustness and ruggedness

The robustness of the proposed method was tested by investigating the effect of using phosphate buffer pH 8 to introduce small changes in pH during the analysis of the tested drug. The proposed sensors remained unaffected by this small variation in method parameters. The calculated percentage recoveries were 98.99±0.68 and 99.63±0.37 for carbon paste and modified carbon nanotubes carbon paste sensors, respectively. These results were closely in agreement with those obtained from standard drug solutions. Also, the reproducibility and the ruggedness of the proposed method were evaluated upon using another model of pH-meter (Jenway 3510). The obtained results were 99.41±0.43 and 99.67±0.33 for both previously mentioned sensors.

3.7.4. Accuracy

The accuracy of the proposed method was investigated by the analysis of glucosamine sulphate in its placebo sample of magnesium stearate using standard addition method. The results obtained in Table 1 showed mean percentage recoveries (99.36±0.34 and 99.72±0.18) for the previously mentioned sensors, respectively revealing good accuracy for the determination of glucosamine sulphate in its pharmaceutical dosage forms.

3.7.5. Precision

The % RSD values for the repeated nine determinations of glucosamine sulphate standard solutions were 0.317% and 0.108% for carbon paste and modified carbon nanotubes carbon paste sensors, respectively. The obtained values of %RSD are less than 2% indicating high precision.

4. CONCLUSION

New fabricated glucosamine carbon paste and modified carbon nanotubes carbon paste sensors were investigated and validated. The use of carbon nanotubes particles to modify the carbon paste one improves the performance characteristics of the sensor in terms of wide linear concentration range, lower limit of detection, fast dynamic response time, high selectivity and reproducibility. Also, the proper choice of plasticizer plays an important role in the improvement of the sensors response in terms of longer life time and sensor stability. From the obtained results it has been shown that the proposed sensors were useful for determination of glucosamine sulphate in bulk powder and its pharmaceutical dosage forms.

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