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Single-Use Probes for the Determination of Dextran Sulfate in Food, Drug, and Industrial Samples

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A simple potentiometric protocol for the assay of the sulfated polysaccharide dextran sulfate (DS) is discussed, based on single-use plasticized poly(vinyl chloride) (PVC) /polyurthane (PU) polyion membrane probes incorporating a quaternary ammonium transducer (tridodecylmethylammonium chloride, TDMAC). A disposable polycationic protamine sulfate (PS) probe, based on dinonylnaphthalene sulfonate (DNNS), is also introduced and used as a tool for end point detection. The DS sensor probes exhibited a detection limit of 0.759 ppm in a 120 mM NaCl background saline solution, a linear range of 0.955 - 4.467 ppm DS, with a slope of -28.1 mV/decade concentration, and an overall potential change of 103.7 mV. The fabricated probes were successfully applied in the determination of DS levels of several energy drink samples, a pharmaceutical formulation, and an industrial polyelectolyte sample.

Keywords: dextran sulfate; potentiometry; polyion probes; food and drug analysis; industrial analysis

1. INTRODUCTION

Dextran sulfate, a polymer of anhydroglucose, is a high molecular weight sulfated polysaccharide sodium salt, produced as the sulfuric acid ester of dextran polysaccharide [1-3]. It is commercially produced from *Leuconostoc* bacteria, with 17% sulfur representing 2.3 sulfate group for each glucosyl residue, and was found to be soluble in aqueous solutions up to 100 mg/mL and can be buffered and sterilized up to 115^{o}C for up to 45 minutes [4, 5].

It has potential applications in biomedical and clinical fields. Oral administration of DS solution induces colitis in mice and rats [6-8] with oral toxicity of 341mg/kg in mice [9]. DS was extensively examined for the in-vitro inhibition of the replication of human immunodeficiency virus (HIV) [10, 11]. 10% DS solution is used in the acceleration of the hybridization of labeled probes with

membrane-immobilized DNA [12], and as an anticoagulant in some medicinal research in Japan [13]. 0.05% DS solution (Av. Mol. Wt. 15000), in the presence of 0.05M $MnCl_2$, is used in the selective precipitation of VLDL- and LDL-cholesterol. Subsequent precipitation of HDL-cholesterol can be carried out by increasing the DS and $MnCl_2$ solution concentrations to 0.65% and 0.2M, respectively [14]. DS reacts with alginate to produce Alginate-Dextran sulfate microgels (ADS) that binds insulin and protects it from gastrointestinal attack, as well as delivering it through intestinal epithelium [15]. Aqueous solutions of DS and polyethylene glycol are used in the separation of bacteria, viruses, proteins, and nucleic acids [16]. Other applications of DS include destabilization in the emulsions of oil-in-water [17], colloid-stabilization for nanoparticles synthesis [18, 19], and in cosmetic products as binder and skin-conditioner [20].

Some of the assay methods for DS depend on the quantitative determination of the sulfate-group content in pharmaceutical products. These methods based on sample combustion, followed by quantitative determination of the produced sulfur oxides [21, 22]. Although their simplicity, such methods lack for adequate sensitivity. Other assay methods include precipitation followed by dielectric permittivity measurements [23], electrophoresis [24], radioactive labeling [25], competitive binding with labeled DS [26], as well as size exclusion chromatography [27]. The latter methods introduce better sensitivity but require sample pretreatment/derivatization steps.

In this article, we introduce a simple, direct, sensitive, and low cost method for the determination of the total dextran sulfate concentration in a variety of samples based on single-use polyion membrane probes and a potentiometric non-equilibrium assay method.

2. EXPERIMENTAL

2.1. Chemicals and reagents

Analytical grade protamine sulfate (PS) (average molecular mass ~ 4500) and dextran sulfate (DS) were purchased from Sigma (www.sigmaaldrich.com). Tridodecylmethylammonium chloride (TDMAC), potassium tetrakis(4-chlorophenyl) borate (KTpClPB), high molecular weight poly(vinyl chloride) (PVC), polyurethane (PU), 2-nitrophenyl octyl ether (NPOE), bis(2-ethylhexyl) sebacate (DOS), and tetrahydrofuran (THF), all are Selectophore grade, were products of Fluka. Dinonylnaphthalene sulfonate sodium salt (DNNS), dioctyl phthalate (DOP), sodium chloride, and potassium chloride were supplied from Aldrich. All other chemicals were of analytical reagent grade unless otherwise stated. Samples containing polyions were obtained from the local market.

100 mL of 0.12 M KCl was prepared by dissolving 0.895 g of KCl in a 100 mL water, and the solution was used as electrodes-filling solution and as sample background working solution. A series of dextran sulfate and protamine sulfate solutions were prepared, with the concentrations of 10 mg/ml, 1mg/ml, and 0.1 mg/ml in 0.12M KCl for calibration experiments. 10 ppm solutions of dextran sulfate and protamine sulfate in 0.12M KCl were prepared for titration experiments.

2.2. Apparatus

Polyion sensor probes, in conjunction with an Orion Ag/AgCl doube-junction reference electrode (Model 90-02) and an Orion digital pH/mV meter (Model SA 720) were used for mV measurements. All aqueous solutions were prepared using bi-distilled de-ionized water obtained from a NANO pure water system (Barnstead model CH-4009, Basel, Switzerland). A Fisher scientific continuously variable speed Vortex mixer was used to mix membrane components during membrane preparations.

2.3. Membrane/probe fabrication

DS polyanion membrane cocktails were prepared with the compositions stated in Table 1. Membranes incorporated different plasticizers (DOP, NPOE, DOS), varying polymeric matrix ratio and composition (PVC, PU), varying ionophore ratio (TDMAC), and with or without membrane additive (KTpClPB), to optimize the best potentiometric response characteristics (Detection limit, linear range and slope). As well, seven PS Polycation membrane formulations were fabricated with different compositions according to Table 1.

Table 1. Different compositions of DS and PS polyion membrane probes

Probe No		Membrane Matrices, mg		Plasticizers, mg			Sensing Element, mg	Membrane Additive, mg
		PVC	PU	DOP	NPOE	DOS		KTpClPB
DS	1	132	0	66	0	0	2 TDMAC	0
	2	132	0	0	66	0	2 TDMAC	0
	3	132	0	0	0	66	2 TDMAC	0
	4	66	66	*Opt.**	*Opt.**	*Opt.**	2 TDMAC	0
	5	0	132	*Opt.**	*Opt.**	*Opt.**	2 TDMAC	0
	6	*Opt.**	*Opt.**	*Opt.**	*Opt.**	*Opt.**	1.5 TDMAC	0.5
	7	*Opt.**	*Opt.**	*Opt.**	*Opt.**	*Opt.**	1 TDMAC	1
PS	8	132	0	66	0	0	2 DNNS	0
	9	132	0	0	66	0	2 DNNS	0
	10	132	0	0	0	66	2 DNNS	0
	11	66	66	*Opt.**	*Opt.**	*Opt.**	2 DNNS	0
	12	0	132	*Opt.**	*Opt.**	*Opt.**	2 DNNS	0
	13	*Opt.**	*Opt.**	*Opt.**	*Opt.**	*Opt.**	1.5 DNNS	0.5
	14	*Opt.**	*Opt.**	*Opt.**	*Opt.**	*Opt.**	1 DNNS	1

**Opt.*: optimized from previous results

Membrane components were dissolved in freshly-distilled THF (100 mg membrane components per 1 ml THF). Single-use polyion sensor probes were fabricated as described earlier by Dürüst and Meyerhoff [28]. Two hours prior to their use, the probes were filled with and soaked in a

0.12 M KCl solution for conditioning. A Ag/AgCl wire was introduced into the tubular sensor before use to serve as an internal reference electrode. All potentiometric measurements were made at room temperature.

2.4. DS and PS Calibrations

Polyanion (or polycation) sensor probes, 3 at a time, in conjunction with the double-junction reference electrode, were inserted in 3 mL of 0.12 M KCl saline solution and baseline potential readings were recorded after stabilization in about 3-5 minutes. Aliquots of 3 - 12 µl of 0.1 mg/mL DS (or PS) calibrants were then added and potential readings were recorded after 5 min of each addition. Calibration is continued by adding 3 - 15 µl aliquots of 1 mg/mL standard calibrant of the subsequent polyion and a calibration graph was plotted between the logarithm of polyion concentration and the cell potential.

2.5. Titrations of DS with PS and vice versa

Pre-conditioned DS (or PS) probes, in conjunction with a double-junction Ag/AgCl reference electrode, were immersed in a 3 mL aliquot of 0.12 M KCl solution with constant stirring. After potential stabilization, a fixed volume (150, 300, or 600 µl) of a 10 ppm standard solution of DS (or PS) was added and the potential readings were recorded after 5 minutes of the addition. Then 25, 50, or 100 µl aliquots of 10 ppm PS (or DS) titrant were added and the corresponding potentials were recorded in 5 minutes after each addition. Titration graphs were then plotted and the corresponding end points were estimated.

2.6. Analysis of real samples

A set of five samples was collected from the local market to contain various levels of DS polyanion. A pharmaceutical formulation sample (obtained from Aseer Central hospital), three energy drink samples, and an industrial polyelectrolyte polyanion sample were collected and their DS levels were determined using DS and PS probes. Samples were diluted 5, 10, and 20 times using 0.12 M KCl solution and the levels of DS in the diluted sample solutions were determined by the aid of DS sensor probes via the standard addition method and through titration against 10 ppm PS solution using PS improved probe (number 8), as well as the PS reference probe.

3. RESULTS AND DISCUSSION

Performance characteristics of DS and PS formulated polyion sensor probes were calculated from calibration experiments and investigated in order to select the best performing probe for each polyion. The selected probes were then used as tools for end-point detection in titration experiments

involving the two oppositely charged polyions in order to calculate their reaction stoichiometry. Afterwards, the optimized probes were applied in the determination of DS polyanionic species in a variety of samples through potentiometric titrations.

3.1. Characteristics of DS probes

DOP, NPOE, or DOS plasticizers were used in the fabrication of DS membrane probes in tubular format (Table 1, probes 1, 2, and 3). It is clear from Table 2 that although the DOP-based probe had the lowest detection limit (0.598 μg/mL), NPOE-based probe showed the longest linear response range (0.724 - 2.570 μg/mL), compared with the other two tested plasticizers (0.741 - 1.995 μg/mL and 1.000 - 2.280 μg/mL for DOP- and DOS-based probes, respectively), with a considerably low detection limit (0.661 μg/mL) and a large overall potential change of 103.7 mV. Accordingly, NPOE was selected as the best plasticizer for DS membrane probes.

NPOE-based DS membranes were prepared using three different matrices, PVC, mixed PVC/PU, and PU, as represented in Table 1 (probes 2, 4, and 5). It is obvious from the results in Table 2 that probe number 4, with 33% PVC/33% PU polymer matrix, showed the best performances in terms of detection limit (0.759 μg/mL), linear range (0.955 - 4.467 μg/mL) and overall potential change (103.7 mV). The possible cause for such behavior could be the increased elasticity of the this PVC/PU membrane, compared with the PVC-based membrane. The much more elasticity of the bare PU-based membrane reduced the response characteristics of the probe, indicating a decrease in the polyanion extraction to the membrane phase. Thus NPOE/(PVC/PU)-based probes were used for all further DS experiments.

Table 2. Potentiometric response characteristics of DS (1-7) and PS (8-14) polyion probes

Probe No.		Detection Limit, μg/mL	Linear Range, μg/mL	Slope, mV/decade	Overall Potential Change, mV
DS	1	0.589	0.741 - 1.995	-49.7	77.6
	2	0.661	0.724 - 2.570	-37.7	80
	3	0.871	1.000 - 2.280	-8.9	14.3
	4	0.759	0.955 - 4.467	-28.1	103.7
	5	0.776	0.955 - 4.266	-17.5	64.8
	6	0.708	0.851 - 2.399	-17.5	32
	7	0.759	2.818 - 3.981	-18.5	62
PS	8	0.513	0.912 - 5.888	21.6	113.5
	9	0.562	1.023 - 5.495	12.8	80.3
	10	0.513	1.000 - 1.995	31.5	103.7
	11	0.524	0.955 - 3.981	14.2	54
	12	0.562	1.096 - 3.631	7.3	25.5
	13	0.646	0.794 - 3.162	19.5	55
	14	0.625	0.668 - 1.479	4.59	3

Addition of the lipophlic additive KTpClPB to the PVC/PU/NPOE-based DS membrane did not affect much on the detection limits of the probes (0.708 and 0.759 μg/mL for sensors 6 and 7, respectively) (Table 2). The presence of KTpClPB diminished the probes' linear ranges, slopes, and overall potential changes in both additive ratios. These findings could be attributed to the repulsion between the negatively charged KTpClPB and the anionic DS that reduces its extraction into the membrane phase and hence deteriorates the probes response.

Accordingly, DS probes were prepared with membrane composition of 66 mg PVC (33%), 66 mg PU (33%), 66 mg NPOE (33%), and 2 mg TDMAC (1%), dissolved in 2 mL THF.

3.2. Characteristics of improved PS probes

PS probes were prepared using DOP, NPOE, or DOS plasticized membranes in the tubular configuration (Table 1, probes 8, 9, and 10). As illustrated in Table 2, the detection limits of the DOP- and DOS-based probes were the same (0.513 μg/mL) and both were slightly better than that of the NPOE-based probe (0.562 μg/mL). On the other hand, the DOP-based probe acquired wider linear range (0.912 - 5.888 μg/mL) and larger overall potential change (113.5 mV) compared with the other two probes. Consequently, DOP plasticizer was used in the preparation of DS membranes for all subsequent experiments.

DOP-based probes were prepared using three different matrices, PVC, PVC/PU, and PU (Table 1, probes 8, 11, and 12). Data in Table 2 indicate that the PVC-based membrane probe (probe 8) acquired the best potentiometric performance characteristics compared with the other two probes (0.513 μg/mL detection limit, 0.912 - 5.888 μg/mL linear range, and 113.5 mV overall potential change). The increase in the PU ratio within the membranes (probes 11 and 12) deteriorated their performances, which can be explained as the increase in the PU ratio increases the membrane elasticity that is not favored for the extraction of PS molecules from the bulk of solution to the membrane phase. Thus DOP/PVC-based probes were used for the following PS experiments.

Effect of incorporating a lipophlic anionic additive to the DOP/PVC-based PS membrane, in order to enhance the probe 's detection limit and linear range, was investigated (Table 1, probes 13 and 14). As indicated in Table 2, the incorporation of KTpClPB diminished the detection limits, the linear ranges, and the overall potential changes of their subsequent probes. This behavior could be attributed to the diminished formation constant of the KTpClPB/PS ion-pair, compared to that of the DNNS/PS ion pair, as the amount of DNNS is reduced and substituted by KTpClPB.

Conclusively, the suggested membrane composition for the improved PS probe contained 132 mg PVC (66%), 66 mg DOP (33%), and 2 mg DNNS (1%), all dissolved in 2 mL THF. The probe showed improved performance, compared with previously reported PS probes (overall potential changes of 50-55 mV and 72mV, and detection limits of 1.6 ppm and 2.5 ppm for references [29] and [30], respectively). Figure 1 represents a combined graph for the calibration relations of DS and PS probes (probes 4 and 8, respectively).

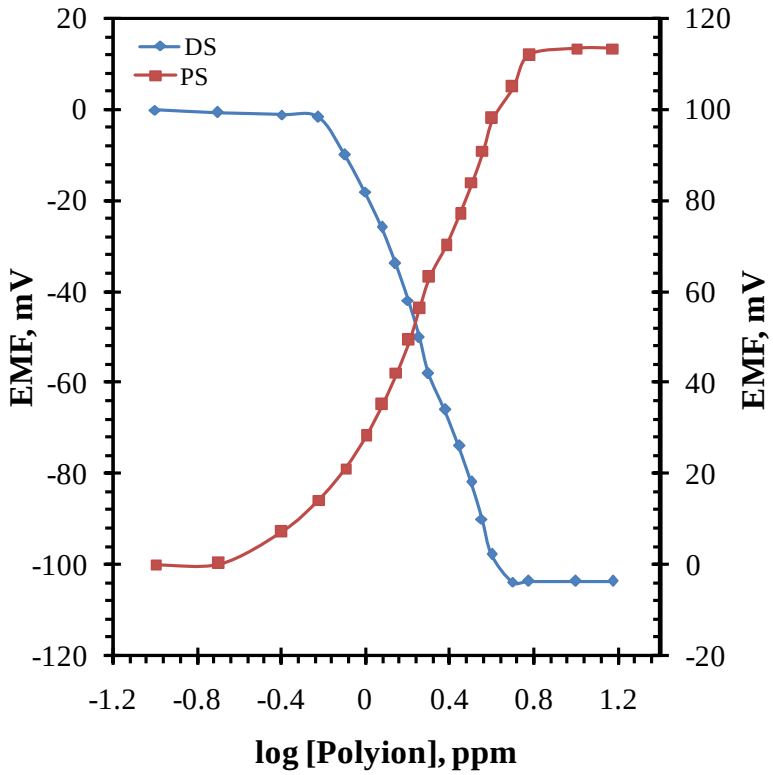

Figure 1. Combined graph for the calibration relations of DS probe (probe 4) and PS probe (probe 8)

3.3. Titrations of DS/PS and PS/DS

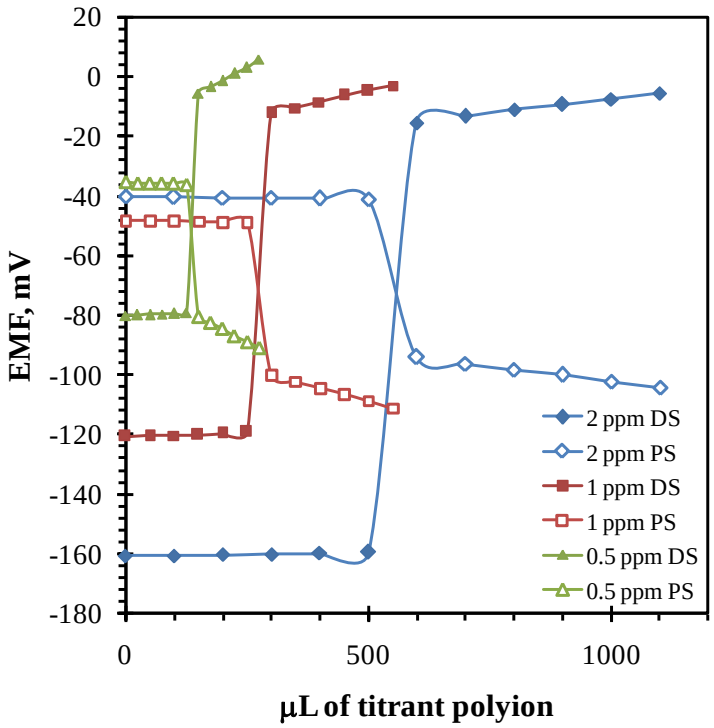

Figure 2. Titrations of 0.5, 1.0, and 2.0 ppm DS against 10 ppm PS using DS probes, combined with titrations of 0.5, 1.0, and 2.0 ppm PS against 10 ppm DS using PS probes

DS standard solutions (0.5, 1, and 2 ppm) were titrated against 10 ppm PS using PS probe (probe 8) and vice versa, where PS standards (0.5, 1, and 2 ppm) were titrated against 10 ppm DS using DS probe (probe 4), in order to calculate the stoichiometry of the ion-pairs formed (Figure 2). From the end-points detection, the calculated mass ratio of the DS/PS complexes was found to equal 1.1:1.0 by the aid of the two probes. This estimated ratio was adapted in the calculation of DS concentrations in real samples via the titration against PS, and using PS probe number 8.

3.4. Real sample analyses

Table 3 summarizes the calculated DS concentrations within the five collected samples using three probes: our investigated DS probe, the improved PS probe, and a reference PS probe (DNNS:PVC:DOS = 1:49.5:49.5 w/w/w, dissolved in 2.5 mL THF) [30]. As can be seen, the data obtained using the three probes via the standard addition and the direct titration methods look insignificantly different from each other. Sample 1 was found to contain an average of 8.45 ± 0.082 ppm DS, which is accurately indistinguishable from the true value indicated by the sample manufacturer (8.5 ppm). DS levels in energy drinks (samples 2, 3, and 4) were adequately determined and are not significantly different using the three probes (Table 3). Such levels (2.59 - 8.65 ppm) can be safely tolerated by human body as it can be administered with DS in doses that reach 45 mg/h through treatment protocols of HIV and other viral diseases [31]. Industrial anionic polyelectrolyte solution sample (sample 5), supplied as a gift from Petrogulf Misr Company, that contained 10 ppm DS polyanion was assessed using the three methods. The data represented in Table 3 (10.24 - 10.57 ppm) are in good accordance with the sample true value.

Table 3. Levels of polyions in real samples

Sample No.	Sample Name	Current DS probe, ppm*	Improved PS probe, ppm*	Reference PS probe [30], ppm*
1	DS pharmaceutical formulation	8.27 ± 0.076	8.53 ± 0.081	8.55 ± 0.088
2	Power Horse	4.63 ± 0.064	4.56 ± 0.077	4.44 ± 0.091
3	Red Bull	8.28 ± 0.046	8.44 ± 0.059	8.65 ± 0.085
4	Bison	2.63 ± 0.059	2.59 ± 0.063	2.82 ± 0.074
5	Industrial Anionic Polyelectrolyte	10.24 ± 0.069	10.39 ± 0.083	10.57 ± 0.096

* number of replicates, n = 3

4. CONCLUSIONS

A Single-use probe for the detection of the polyanion dextran sulfate was developed with significant response characteristics. An improved protamine sulfate polycationic membrane probe was also investigated. The two probes, in comparison with a reference protamine sulfate probe, were

successfully applied in the analysis of dextran sulfate levels in several real samples. The obtained data were not significantly different, and in good accordance with samples true values and human body tolerable levels.

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