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Note

Rapid quantitative determination of the sulphate groups of dextran sulphate

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Abstract

We describe a method for the determination of sulphate groups of dextran sulphate by precipitation with a standard solution of an organic amine compound. The method used to determine the dosage equivalence point is new and based on the measurement of the medium dielectric permittivity during titration. The method has been compared to a previous and classical procedure based on sample combustion followed by coulometric titration of the sulphur dioxide released. This new method is easy, rapid, can be automatized and presents a limit of detection of 10^{-4} M (expressed in sulphate concentration). In addition, this method has applications in raw material control as well as in formulation.

Keywords: Dextran sulfate determination; Dielectric end-point determination

Dextran sulphate (DS) has been used clinically as an anticoagulant or antilipemic agent especially in Japan (Mitsuya et al., 1988). DS activity against HIV has been demonstrated in vitro (Mitsuya et al., 1988; Nakashima et al., 1989) and clinical trials have already been carried out in the treatment of AIDS (Abrams et al., 1989). As an adjuvant in formulation, DS is used as a stabilizing agent, particularly for colloidal carriers such as nanoparticles (Marchal-Heussler et al., 1990; Benoit et al., 1994).

As the activity of DS, both in therapeutics and as a stabilizing agent, is linked to the presence of sulphate groups carried by the D-glucose polymer, it would be particularly interesting to have a rapid quantitative determination method of these sulphate groups in order to measure the sulphation level of commercial samples as well as to control pharmaceutical preparations.

The methods used so far are generally based on sample combustion which is followed by a dosage, using different methods, of the mineral sulphur released in the medium (Kirsten et al., 1963; Debal and Levy, 1968). A few other techniques involve the direct titration of sulphate groups by volumetric analysis, but other mineral ions present in commercial samples of DS (for example phosphate or sodium ions) may interfere

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and these methods may lack sensitivity (Charlot, 1974).

In biology, there are also other methods such as either electrophoresis (Jaques et al., 1990), radioactive labeling (Foster et al., 1990), or competitive binding methods with radiolabeled DS (Lorensen et al., 1989). However, all of these methods allow for the determination of the total amount of DS, and not for that of the sulphate groups. An interesting size-exclusion chromatographic method has already been described, however, it needs a post-column derivation reaction (Maderich and Sugita, 1993).

The aim of the present report is to propose a very simple dosage method in order to determine the quantitative sulphation level of DS, and applicable in pharmaceutical control. It is based on a precipitation reaction of DS by amines such as propranolol and cetylpyridinium. Such a reaction between acidic polysaccharides and amines has been described previously by Scott (1960). What is completely new in the determination method we propose is the end-point determination based on the measurement, at high frequency, of the dielectric permittivity of the reaction medium during titration. As a control, the results obtained with the former method have been compared with a classical method of combustion followed by the determination of sulphur dioxide by acidimetry (Debal and Levy, 1968).

Dextran sulphate 500 000, 50 000, 5000, and cetylpyridinium chloride (Sigma, France) and propranolol hydrochloride (Cooper, France), were used without further purification.

Solutions of propranolol hydrochloride and of cetylpyridinium chloride were previously standardized by an argentometric measurement of their chloride concentration followed by a potentiometric end-point detection.

The dosage is based on the quantitative precipitation reaction (resulting in a white precipitate) of stoichiometry (1:1) occurring between the sulphate groups of DS and the amine group of either propranolol or cetylpyridinium.

A minimal volume of 6 ml of an aqueous solution of DS with concentration ranging approximately between 10^{-4} and 5×10^{-3} M is titrated in a capacitive cell exposed to a high-frequency field (10 MHz), by an aqueous solution of propranolol or cetylpyridinium hydrochloride of concentration ranging from 0.01 to 0.1 M poured from a 2 ml microburette. The solution is mixed in the cell using a motor-rotating helix plunging in the sample (magnetic agitation must not be used due to the end-point detection method). The complex impedance and phase shift at 25°C are measured throughout the titration, according to a previously described method (Thiébaut et al., 1989) with a Hewlett Packard 4194 A vector impedance/gain-phase analyzer.

With the help of a physical model, the measured values are computed to obtain the dielectric permittivity, ϵ', which is plotted vs the titrating reagent volume to determine the dosage end-point.

The dextran sulphate sample is burned under an oxygen stream between 1320 and 1360°C. The sulphur is transformed into sulphur dioxide, and titrated in an acidimetric coulometric cell. This method has been described in detail earlier (Debal and Levy, 1968).

The dielectric measurements carried out at high frequencies lead both to ϵ', the dielectric permittivity, and to ϵ'', the dielectric loss. However, only ϵ' displays the end-point of the titration.

The frequency chosen for these measurements was 10 MHz: this frequency is higher than the relaxation frequency zone and yields more reproducible values relative to other frequencies (Ishikawa et al., 1984).

For propranolol hydrochloride or cetylpyridinium chloride, the titration curves look similar (Fig. 1): they present two linear segments whose intersection corresponds to the end-point. The first linear part shows the decrease of the dielectric permittivity, corresponding to the disappearance of DS due to its reaction with the amine reagent. The second flat part indicates that there is no more free DS in solution and therefore that the determination is completed. This aspect can be explained by the fact that the dielectric permittivity is much more sensitive to the presence of charged polymers of high molecular weight (as DS) than to the presence of ions of smaller size (such as propranolol or cetylpyridinium). This

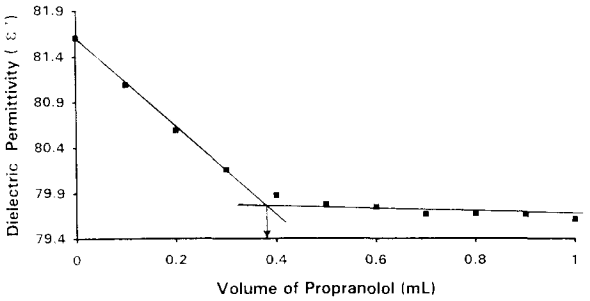

Fig. 1. Titration curve of a solution of DS by a standard solution of propranolol.

hypothesis has been verified by following the value of the dielectric permittivity vs the concentration of DS and propranolol (Fig. 2): for DS, the ϵ' value increases with concentration in contrast to propranolol whose value stays constant. Fig. 2 also demonstrates that it is better to carry out the determination with a DS initial concentration lower than 5×10^{-3} M, in order to be in the quasi-linear segment of the DS curve. Indeed, under such conditions, the detection curve is the intersection of two linear segments. The volume of the titrating compound at the equivalence point (Fig. 1) is given by the abscissa of the intersection of the two straight lines whose equations have been determined by linear regression.

Working at 10 MHz, at 25°C and with a 25 ml sample, it is possible to detect sulphate concentrations as low as 10^{-4} M (i.e., 500 μg as the minimal quantity of DS). The quantification limit can be lowered working at 0.1 MHz but unfortunately this also affects the precision of the method.

The relative coefficients of variation ($n = 6$) determined for 15 ml of a 10^{-3} M DS 500000 solution with cetylpyridinium 0.037 M and propranolol 0.038 M at 10 MHz are 5.5 and 4.1%, respectively.

The determination has been carried out on the same commercial sample, by the SO_2 determination method in order to validate the proposed method. It should be mentioned that the reference determinations have been performed by an independent laboratory (Service central d'analyse du Centre National de la Recherche Scientifique, Vernaison, France).

The mean result obtained after six determinations leads to a difference of +1.3 and +0.9% compared to the reference method in which propranolol and cetylpyridinium are the reagents, respectively.

The reference method gives a variation coefficient of 4.8%, showing that the variation coefficient obtained by our method is of the same magnitude. In both cases, the high values obtained for the coefficients of variation may be due either to some heterogeneity of the commercial samples or to the considerable hydration of these types of products.

For the quantification limit, the reference method is less sensitive (9×10^{-2} M instead of 10^{-4} M).

The proposed method allows one to determine DS concentrations in solution as low as 10^{-4} M (expressed as sulphate). Although one might consider the method to be more successful using high rather than low molecular weight DSs, it is interesting to note that different molecular weights of DS, i.e., 5000, 50000 and 500000 work equally well.

This type of precipitation reaction has already been proposed for determining the concentrations of acidic polysaccharides but the end-point was determined by either a visual method or by indirect spectrophotometry (Scott, 1960). Dielectric permittivity measurement is a satisfactory way of evaluating the end-point, since it is linearly related to the disappearance of DS. Furthermore, after the end-point, when there is no more free DS, the ϵ' value stays constant.

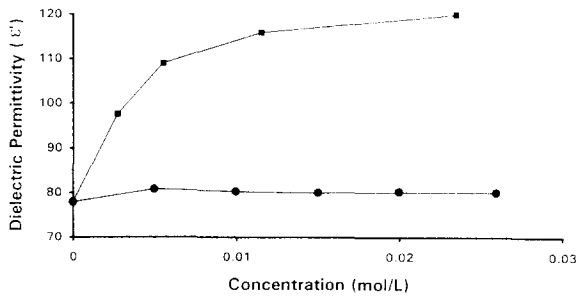

Fig. 2. Dielectric permittivity vs DS and propranolol concentrations in aqueous solution: (■) DS, (●) propranolol.

For this determination, we also attempted to use classical conductimetry and nephelometry techniques to detect the end-point, however, both methods failed to detect the end-point, i.e., the measured values did not present any peculiar variation displaying the equivalence point.

In a related field, dielectric permittivity has previously been used to study the adsorption of some drugs onto colloidal systems. Indeed, by measuring the dielectric permittivity of suspensions of polybutylcyanoacrylate nanoparticles in the presence of DS, it has been found possible to follow the in situ adsorption of various β-blockers onto the particles without any need for either a separation step or analytical determination of the drugs (Benoit et al., 1992, 1994).

One of the major advantages of the reported method is that it is rapid and very easy to carry out. In addition, no special knowledge is required. Furthermore, the reagents, propranolol as well as cetylpyridinium, are inexpensive and readily available.

On the other hand, the major drawback of this method is the lack of selectivity as other sulphate groups eventually present in a sample can interfere: however, we verified that the presence of inorganic sulphate and phosphate ions in the commercial samples did not interfere with the dosage probably because of their low molecular weights.

Furthermore, this method is not sensitive enough to determine DS in biological fluids.

This new method can give interesting results in the control of raw materials and particularly for the determination of sulphation levels. The present technique is also useful for measuring the concentrations of sulphate groups in pharmaceutical formulations. For example, it is possible to measure the amount of DS (used as a stabilizing agent) linked onto nanoparticles. We have also shown that it is possible to measure, without any interference, DS concentration even when dextran 70000 is present. Finally, the developed technique could be completely automatized which is not the case for other methods of determination of the end-point based on the same titration reaction (Scott, 1960).

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