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Deactivation of Sodium Tetradecyl Sulphate Injection by Blood Proteins

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Abstract *Objectives:* The aim of this work was to quantify the volume of blood required to deactivate 1 ml of 3% sodium tetradecyl sulphate (STS).
Design: A series of experiments were performed where the concentration of STS remaining in a stock solution was measured after adding increasing volumes of blood protein solutions.
Materials and methods: Increasing volumes of bovine serum albumin, bovine erythrocytes and a mixture of both was added to a stock solution of STS. The BP manual titration method was used to measure the assay of the remaining STS.
Results: The method was reproducible and increasing volumes of blood protein lowered the STS concentration in a linear fashion. Approximately 2 ml of a 4% blood protein solution deactivates 1 ml of 3% STS, which means approximately 0.5 ml of whole blood will deactivate 1 ml of 3% STS.
Conclusions: Sodium tetradecyl sulphate injection is deactivated by a relatively small volume of blood. The practical implication is that changes in technique to reduce the blood volume in larger veins and to introduce fresh aliquots of sclerosant along the length of the vein could improve the efficacy of sclerotherapy.

Introduction

Injection into a blood vessel was first described by Pravaz in 1853 when he injected ferric chloride into an aneurysm. Over the next hundred years many different sclerosing agents were tried for the treatment of varicose veins with varying degrees of success.[1] The use of sodium tetradecyl sulphate (STS) as a sclerosant was described in 1946 and its use soon became popular.[2] Sclerosants containing STS as the active ingredient are now widely used around the world.

Irish surgeon George Fegan published the results of his 'Continuous compression technique of injecting varicose veins' using STS in 1963.[3] He had a fastidious technique and was especially particular that the vein to be treated should be free of blood, often referred to as 'the empty vein technique'. From *in-vitro* work he concluded that STS binds with plasma proteins and is deactivated, consequently the section of vein to be treated should be free of blood.[4]

Following initial enthusiasm, the use of sclerotherapy to treat large varicose veins and truncal veins declined and

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a study showed that the long term results of surgery were more robust.[5] It was thought that in larger veins the liquid sclerosant mixed with the blood was diluted, deactivated and unable to damage the endothelium.

Some elegant *in vitro* work by Parsi and colleagues confirmed Fegan's belief that the action of STS is indeed inhibited by blood proteins.[6] They studied the effect of increasing concentrations of sclerosant on cultured endothelial cells and red blood cells with and without the addition of albumin or blood plasma. A higher concentration of STS was required to damage the cells in the presence of blood proteins.

It is clear that blood proteins deactivate STS. However the effect of whole blood on working concentrations of STS injection has not been established. Therefore the aim of this study was to quantify the volume of blood required to neutralise 1 ml of 3% STS injection.

Materials and Methods

There are two in house methods at STD Pharmaceutical Products for measuring the concentration of sodium tetradecyl sulphate in solution. One method involves the use of an auto-titration machine; the other method is to perform a standard manual titration as listed in the British Pharmacopeia for sodium tetradecyl sulphate concentrate and injection.[7]

In pilot experiments to develop a reliable method of measuring the residual concentration of STS in solution after the addition of protein, increasing volumes of a 1.6% bovine serum albumin (BSA) solution were added to a stock solution of 20 ml of 0.12% STS and then the concentration of the STS in the mixture was measured.

The auto-titrator method was used first and gave readings when a small amount of the BSA solution was added. As more BSA solution was added it became impossible to obtain an end reading, probably because the protein was interfering with the probe.

The next pilot experiment used the manual titration method as described in the BP monograph for sodium tetradecyl sulphate injection. A stock solution of 20 ml of 0.12% STS was prepared in a conical flask and to this solution an indicator solution was added (chloroform and dimidium bromide—sulphan blue solution). The indicator solution changed from pink (Fig. 1) to grayish blue (Fig. 2) as the STS was deactivated by the addition of hyamine (0.004 m) from a burette. The concentration of STS in the solution was calculated by measuring the amount of hyamine required to deactivate the STS. Each ml of hyamine being equivalent to 1.266 mg of STS. Five titrations were made where increasing volumes of a 1.6% BSA solution was added to the stock STS solution.

The end point colour change from pink to grey/blue was more difficult to read as the albumin levels increased but the results gave good linearity in the pilot experiments.

Three experiments using the BP manual titration method were performed to quantify the concentration of free STS remaining in solution after the addition of:

1. increasing volumes of a BSA solution
2. increasing volumes of a solution of reconstituted dried bovine erythrocytes
3. increasing volumes of a simulated blood solution.

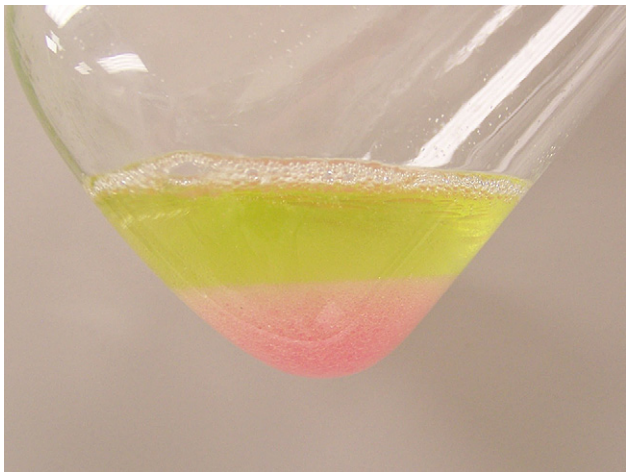

Figure 1 Start point of titration the indicator is pink.

Exp. 1. The BSA solution was made from albumin bovine serum lyophilised powder (Sigma Aldrich product number A9647 Batch No. 076K0717) dissolved in de-ionised water at a concentration of 1.6% w/v. A BSA concentration of 1.6% was used because it gave a good working range in pilot experiments. The stock solution of 0.12% STS was made from 10 ml of 3% Fibro-Vein, batch 7K032, in 250 ml of de-ionised water.

Exp. 2. The erythrocyte solution was made by dissolving dried bovine erythrocytes (Sigma Aldrich product number H3760 Batch No. 077K0676) in de-ionised water at a concentration of 1.6% w/v.

Exp. 3. The third experiment involved making a solution of BSA and erythrocytes to mimic a blood solution. It was assumed that blood comprises of approximately 20% protein and a simulated laboratory blood was made up of 4% albumin plus 16% erythrocytes. The solution was then diluted to give an overall protein concentration of 1.6% for the titration. The batch of Fibro-Vein used to create the stock STS solution was 8B046.

For each of the experiments individual titrations were made following the addition of 0, 1, 2, 3 and 4 ml of one of the protein solutions to a fresh solution of 0.12% STS. The titration began approximately 15 s after adding and mixing the protein solution to the STS solution.

Figure 2 End point of titration the indicator turns blue.

For the BSA and erythrocyte solutions three titrations were made for each point. For the blood solution four titrations were made for each point.

Statistical methods

The average titre result for each volume of protein solution added and the standard deviation were calculated using Microsoft® Excel 2003. The same software was used to plot the results and to calculate the best fit trend line as well as the correlation coefficient between the volume of protein added versus average titre result. The error bars on the graphs are +/− one standard deviation.

Results

Experiment 1

The addition of albumin caused some precipitation in the solution but end points were quite clear. A plot of the average result of three individual titrations for each point is presented in Fig. 3.

The linear trend line has a correlation coefficient off −0.998. Extension of the trend line gives an endpoint of 4.23 ml of 1.6% albumin to neutralise all of the STS.

Thus 42.3 ml of 0.16% albumin solution would neutralise 20 ml of 0.12% STS solution, which is the equivalent of 2.12 ml of 4% BSA to neutralise 1 ml of 3% STS. The result has been converted to 4% BSA out of practical interest because it is the concentration of albumin in blood.

Experiment 2

Increasing volumes of the erythrocyte solution caused quite a lot of precipitation and the end points were more difficult to determine which can be seen in the error bars on the graph. A plot of the average result of three individual titrations for each point is presented in Fig. 4.

Despite the difficulty determining the end points the combined results gave a linear trend line correlation coefficient off −1.000. Extension of the trend line gives an endpoint of 4.79 ml of 1.6% erythrocyte solution to neutralise all of the STS.

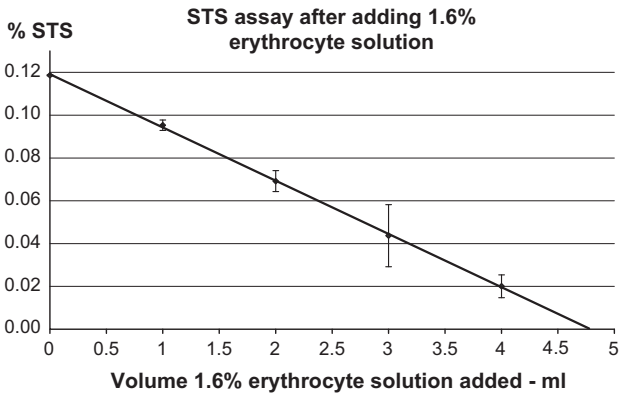

Figure 4 STS assay after adding increasing volumes of an erythrocyte solution to a stock STS solution.

Thus 47.9 ml of 0.16% erythrocyte solution neutralises 20 ml of 0.12% STS solution, which is the equivalent of 2.40 ml of 4% erythrocyte solution to neutralise 1 ml of 3% STS.

Experiment 3

The addition of a laboratory blood solution caused quite a lot of precipitation in the solution and the end points were more difficult to determine which can be seen in the error bars on the graph. A plot of the average result of four individual titrations for each point is presented in Fig. 5 (NB titrations 3 and 4 were performed the day following titrations 1 and 2).

The linear trend line has a correlation coefficient of −0.998. Extension of the trend line gives an endpoint of 4.29 ml of 1.6% lab blood to neutralise all of the STS.

Thus 2.15 ml of 0.16% lab blood solution neutralises 20 ml of 0.12% STS solution, which is the equivalent of 2.15 ml of 4% lab blood to neutralise 1 ml of 3% STS.

A summary of the results is shown in Table 1 in terms of the volume of a 4% protein solution required to neutralise 1 ml of a 3% STS solution.

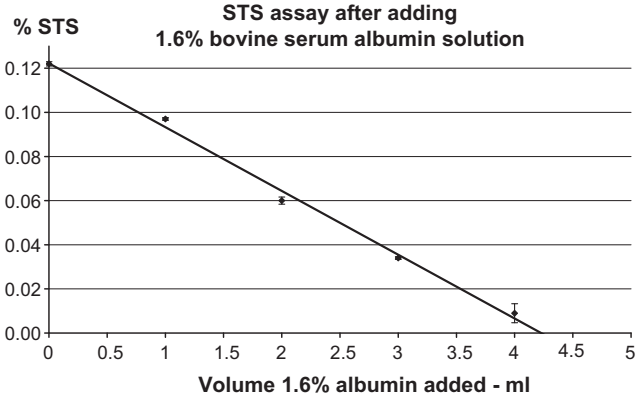

Figure 3 STS assay after adding increasing volumes of BSA to a stock STS solution.

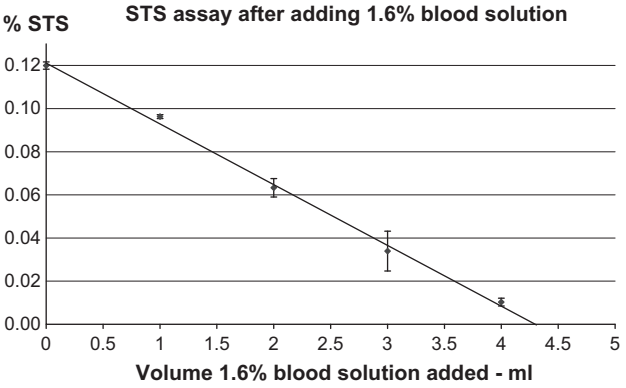

Figure 5 STS assay after adding increasing volumes of a laboratory blood solution to a stock STS solution.

Table 1 Volume of protein solution required to neutralise 1 ml of 3% STS injection.

Protein solution added	Volume
4% bovine serum albumin	2.12 ml
4% bovine erythrocytes	2.40 ml
4% laboratory blood	2.15 ml

If we extrapolate the result for the laboratory blood, at 4% protein, to whole blood at approximately 20% protein the results suggest that only 0.43 ml of whole blood would be required to neutralise 1 ml of 3% STS.

Discussion

The results show that STS is deactivated by blood proteins in a linear fashion. The protein source (bovine serum albumin or red blood cells) seemed to make little difference with just over 2 ml of a 4% protein solution deactivating 1 ml of a 3% STS solution.

It is assumed the titration measured the free STS remaining in solution after the addition of a blood protein which would bind and deactivate some of the STS. The repeatability and linearity of the results would suggest that this is a reasonable assumption but nonetheless it could be a source for error. The main source of error was visually determining the end point of the titration with increasing volumes of blood proteins which is why the average of three (or four) individual readings was used. The results should perhaps be used as a guide rather than an exact figure.

What these results suggest is that in practice it would take less than 0.5 ml of whole blood to deactivate 1 ml of 3% STS, which would explain why an empty vein technique is important when injecting liquid sclerosant into varicose veins.

Parsi et al. investigated the effects of BSA and human blood plasma on the lytic effects of STS on red blood cells, platelets and cultured endothelial cells.[6] Their results showed that a 33 fold increase in the concentration of STS was required to lyse red blood cells in the presence of BSA versus just saline. The addition of whole blood required a 50 fold increase in the STS concentration. This work showed that BSA and other blood proteins deactivate STS and supports the findings of Parsi et al.

They concluded that 5 ml of blood would have enough plasma proteins to neutralise 1 ml 3% STS. This work concludes that just over 2 ml of 4% BSA would do the same thing.

The main difference between the work of Parsi et al. and this work is that they were measuring the lytic effects of sclerosants on blood and endothelial cells in the presence of blood protein whereas this work quantifies the residual STS remaining after the addition of protein to a standard STS solution. Given the differences in the experimental methods it is difficult to draw direct comparisons between the results. However taking both sets of results it is clear that a relatively low volume of blood (0.5—1 ml) is enough to neutralise 1 ml of 3% STS.

In his book, 'Varicose Veins: Compression Sclerotherapy', Fegan refers to the results of some experiments on the action of STS and quotes '*Addition of STS to serum proteins caused turbidity, and subsequent electrophoresis showed abnormal migration of the protein fractions. The reaction, which is very rapid, appears to be a combination of STS and plasma protein. As a result there is a complete inhibition of the detergent properties of the sclerosant*'.[4] The work by Parsi et al. and the results of these experiments support Fegan's findings.

What practical implications do these results have? Studies have shown that ultrasound guided foam sclerotherapy is more effective than liquid sclerotherapy.[8] It is generally believed that the foam displaces the blood better than liquid which mixes with the blood and becomes deactivated. However foam sclerotherapy becomes less effective as veins get larger.[9—11] Larger veins can be treated but it may take more sessions. It seems likely that as the veins get larger foam is less effective at displacing blood and the sclerosant becomes deactivated as it does in liquid form.

The results suggest that practical techniques for reducing the blood volume in a vein as well as introducing fresh sclerosant along its length may have an advantage particularly when treating larger veins. For example, the use of tumescent compression around a truncal vein to reduce the blood volume and introducing sclerosant along its length via a catheter.[12,13]

Conclusion

Sodium tetradecyl sulphate injection is deactivated by a relatively small volume of blood, 1 ml of 3% STS is deactivated by less than 0.5 ml of a simulated laboratory blood. The practical implication is that changes in technique to reduce the blood volume in larger veins and to introduce fresh sclerosant along the length of the vein could improve the results of sclerotherapy.

Conflict of Interest/Funding

The author is employed by STD Pharmaceutical Products Ltd.

The work was carried out in the laboratory at STD Pharmaceutical Products Ltd.

STD Pharmaceutical Products manufacture and market a sodium tetradecyl sulphate based sclerosant called Fibro-Vein.

References

1 Imhoff E, Stemmer R. Classification and mechanism of action of sclerosing agents. *Phlebologie* 1969;**22**:143—6.
2 Reiner L. The activity of anionic surface active compounds in producing vascular obliteration. *Proc Soc Exper Biol Med* 1946; **62**:49—54.
3 Fegan WG. Continuous compression technique of injecting varicose veins. *Lancet* 1963;**11**:109—12.
4 Fegan G. *Varicose veins: compression sclerotherapy.* London: Heinemann Medical; 1967.
5 Hobbs JT. Surgery or sclerotherapy for varicose veins: 10-year results of a random trial. In: Tesi M, Dormandy JA, editors. *Superficial and deep venous diseases of the lower limbs.* Turin: Panminerva Medica; Sept 1984. p. 243—8.
6 Parsi K, Exner T, Connor DE, Herbert A, Ma DD, Joseph JE. The lytic effects of detergent sclerosants on erythrocytes,

platelets, endothelial cells and microparticles are attenuated by albumin and other plasma components in vitro. *Eur J Vasc Endovasc Surg* 2008;**36**:216—23.
7 *British pharmacopoeia*. Clifton House, Worship Street, London, EC2A 2EJ: By British Pharmacopoeia Commission. The Stationary Office Ltd; 2009.
8 Ouvry P, Allaert FA, Desnos P, Hamel-Desnos C. Efficacy of polidocanol foam versus liquid in sclerotherapy of the great saphenous vein: a multicentre randomised controlled trial with a 2-year follow-up. *Eur J Vasc Endovasc Surg* 2008;**36**: 366—70.
9 Barrett JM, Allen B, Ockelford A, Goldman MP. Microfoam ultrasound-guided sclerotherapy treatment for varicose veins in a subgroup with diameters at the junction of 10 mm or greater compared with a subgroup of less than 10 mm. *Dermatol Surg* 2004;**30**:1386—90.
10 Myers KA, Jolley D, Clough A, Kirwan J. Outcome of ultrasound-guided sclerotherapy for varicose veins: medium-term results assessed by ultrasound surveillance. *Eur J Vasc Endovasc Surg* 2007;**33**:116—21.
11 Gonzalez-Zeh R, Armisen R, Barahona S. Endovenous laser and echo-guided foam ablation in great saphenous vein reflux: one-year follow-up results. *J Vasc Surg* 2008;**48**:940—6.
12 Parsi K. Catheter-directed sclerotherapy. *Phlebology* 2009;**24**: 98—107.
13 Cavezzi A, Tessari L. Foam sclerotherapy techniques: different gases and methods of preparation, catheter versus direct injection. *Phlebology* 2009;**24**:247—51.