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Scale-up synthesis of mesna using alkyl trithiocarbonate approach

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ABSTRACT

Mesna is in a class of drugs known as chemoprotectants. Mesna is used for the prevention of urothelial toxicity in patients being treated with the antineoplastics ifosfamide or cyclophosphamide. In this study, a new method for synthesis of mesna from 1,2-dichloroethane using trithiocarbonate salt intermediate was continuously developed by the scale of 100g per batch. The synthesis was carried out in 3 steps: Firstly, sodium 2-chloroethanesulfonate was prepared from 1,2-dichloroethane by Strecker reaction. Secondly, sodium 2-chloroethanesulfonate was reacted with sodium trithiocarbonate, followed by acidification to pH 1.43 by H_2SO_4 or HCl solution. Finally, mesna was obtained by adjusting pH to 6.6 with 1.5 M NaOH solution. The product was purified by crystallization from 96% ethanol. The overall yield of the synthesis process from 1,2-dichloroethane was 38.85 % based on sodium sulfite. The structures of the compounds were defined by IR, MS and NMR spectra data analysis. The product has met all quality requirements of British Pharmacopoeia 2015 therein the content of dried substance was 98.8%.

1. INTRODUCTION

Mesna (brand names: Mesnex, Uromitexan) is an important synthetic compound that protects the bladder from the urotoxic metabolites of oxazaphosphorine antineoplastics (ifosfamide, cyclophosphamide) by chemically interacting with them and their metabolites. In this connection, mesna binds to toxic acrolein within the urine[1-3]. The free sulfhydryl group combines directly with the double bond of acrolein as well as other urotoxic 4-hydroxyoxazaphosphorine metabolites. This medication is given as an infusion into a vein (intravenous) with or after chemotherapy. Mesna may also be given as a pill to be taken by mouth[3].

Chemically, this molecule has a fairly simple structure which consists of two groups: thiol (-SH) and sulfonate ($-SO_3Na$) linked together via the ethylene bridge ($-CH_2-CH_2-$) (Figure 1.)

The literature has shown that mesna can be synthesized by different pathways. The reported synthetic methods for preparing mesna consist of two main stages, namely the formation of $-SO_3$ Na group (by the Strecker reaction of 1,2-halogenoethane and Na_2SO_3) and the building of -SH group (by hydrolysis or reduction various intermediate functionalities, such as thioester, thiouronium, alkyl-xanthate and Bunte salt)[2, 4-8].

Recently, we have found the novel approach to synthesis of this drug in the laboratory scale (~1 g/experiment) from 1,2 -dihalogenoethane by using alkyl trithiocarbonate intermediate. This new method has produced mesna to satisfy all the requirements of British Pharmacopoeia 2015 (BP 2015) in the total yield up to 44.44% and without using ion-exchange chromatography to purify, which is applied by necessity in the other routes[9]. Our method has strong potential for practical preparing of mesna

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in lager scale. So the aim of this study was to expand the synthesis process (up to 100 gram per batch) to obtain mesna satisfied all required standards of BP 2015.

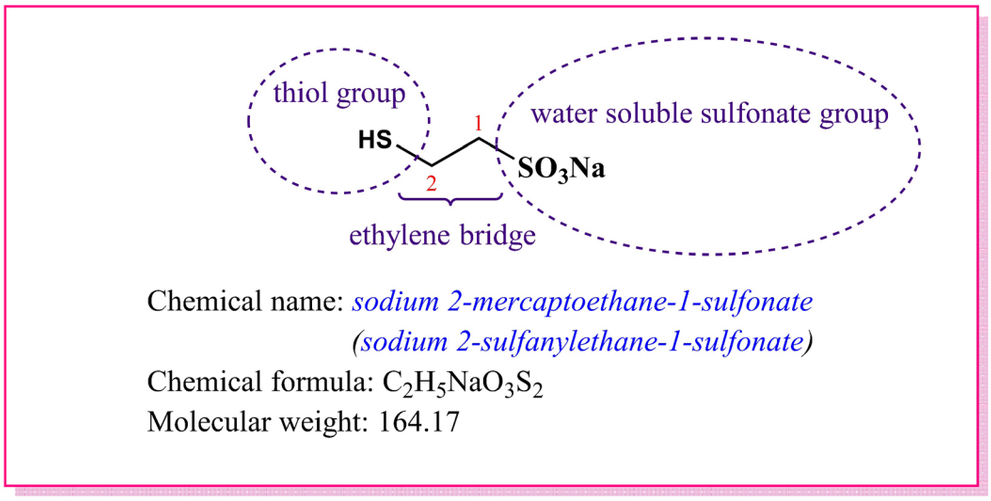


Figure 1. Chemical structure of mesna

2. MATERIALS AND METHODS

Materials: All chemicals used for synthesis were in AR (analytical reagent) grade: 1,2-dichloroethane, sulfuric acid, hydrochloric acid (37 wt. % in H_2O), sodium hydroxide, sodium sulfite, sodium trithiocarbonate, copper powder, 96% ethanol.

The synthesis process was performed by 3 steps from 1,2-dichloroethane (1) as shown in Figure 2, namely, Strecker reaction of 1 with sodium sulfite to obtain sodium 2-chloroethanesulfonate (2), S-alkylation of 2 by sodium trithiocarbonate and hydrolysis using sulfuric acid in one-pot procedure to prepare acid 2-mercaptoethanesulfonic, followed by neutralization at pH of 6.6 by sodium hydroxide to afford final mesna (4)[10].

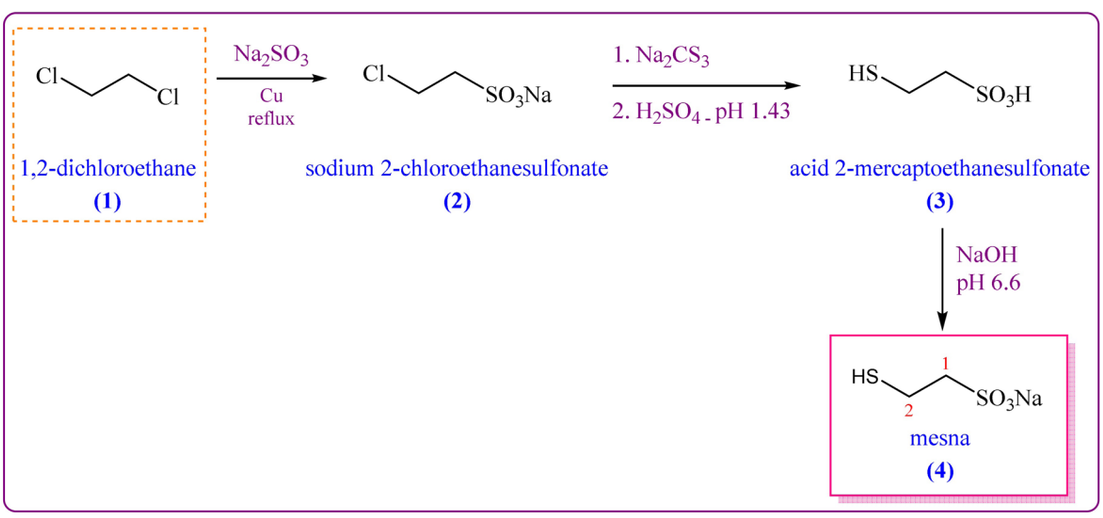


Figure 2. Synthesis scheme of mesna from 1,2-dichloroethane by alkyl trithiocarbonate approach

The reactions were monitored by thin layer chromatography (TLC) using precoated silica gel 60 F254 plates (Merck) and suitable mobile phase solvent system: *n*-butanol : acetic acid : water (9.0 : 2.0 : 2.5; v/v). Spots on TLC were detected under UV light (254 nm) or by spraying with 10% sulfuric acid in ethanol followed by heating. The reaction products was purified by crystallization in 96% ethanol. Melting points were determined on EZ-Melt Automated Melting Point Apparatus (USA). pH values were measured by Mettler Toledo pH meter.

The structure was analyzed by spectral methods, including infrared spectroscopy (IR), mass-spectrometry (MS) and nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR). IR spectra were recorded on the Perkin Elmer machine in the region of 4000-400 cm^{-1}. The mass spectrum was obtained in an electrospray ionization (ESI) mode using Agilent LC/ MS/MS-Xevo TQMS and S1100 Series LC/MSD Trap spectrometer. NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer.

The synthesized mesna was tested in accordance with the standards of monograph "Mesna" in BP 2015, herein the content assay is performed by volume titration using iodine and sodium thiosulphate[11].

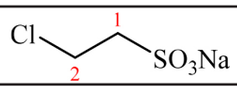
3. RESULTS

3.1 Synthesis and exploration of synthetic process on a scale of 10 g per batch

3.1.1 Preparation of sodium 2-chloroe thanesulfonate

A mixture of 1,2-dichloroethane (36.0 mL, 0.46 mole) and sodium sulfite (19.0 g, 0.15 mole) was dissolved in mixed solvents of 96% ethanol (147 mL) and water (205 mL) in a two-necked flask. Copper powder (0.8 g, 12.5 mmole) was added. The reaction mixture was stirred, heated under reflux (about 70 °C) by a water or parafin bath for 22 hours. Then, copper powder was filtered off from the mixture by hot filtration regime, washed with hot 96% ethanol. The filtrate was collected and concentrated under vacuum to dryness. 220 mL of 96% ethanol was added to the solid mixture, heated to the boil. The obtained suspension was filtered by hot condition. The filtrate was cooled to room temperature and let crystallization overnight to afford 19.90 g (yield 79.65 % calculated according to sodium sulfite) of sodium 2-chloroethanesulfonate with melting point (mp.) 293-295 ^{o}C and spectroscopic data as shown in Table 1.

Table 1. IR, MS, ^{1}H-NMR and ^{13}C-NMR data analysis of sodium 2-chloroethanesulfonate

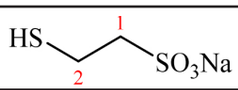
Structure	
IR (KBr): $\bar{\upsilon}$, cm^{-1}	1165 and 1038 (-SO_3^-), 799 and 764 (C-Cl)
ESI-MS (MeOH): *m/z*	143.06 [M-Na]$^-$ ($C_2H_4ClNaO_3S$, M=166.55)
^{1}H-NMR (D_2O, 500 MHz): δ, ppm	3.38-3.41 (2H, m, H-1); 3.89-3.92 (2H, m, H-2)
^{13}C-NMR (D_2O, 125 MHz): δ, ppm	37.96 (C-2); 52.88 (C-1)

3.1.2 Preparation of mesna

In a 250 mL round bottom flask, sodium 2-chloroethanesulfonate (20 g, 0.12 mole) was added to 40 % solution of sodium trithiocarbonate (51.23 mL, 0.18 mole). The mixture was stirred and kept at 55 ^{o}C for 5 hours. After required time, the aqueous solution evaporated to dryness. The residue was added with 50 mL absolute ethanol. A rapid filtration was carried out to obtain precipitate which was then suspended in water (20 mL) to obtain a suspension. A solution of sulfuric acid 35 % or concentrated hydrochloric acid was added dropwise to the suspension and stirred until pH was to be 1.43. The reaction was

maintained for 20 minutes then aqueous sodium hydroxide 1.5 M was added until pH was to be 6.6. In both pH-adjusted steps, a continuous stirring and supplying of nitrogen gas were necessary and important. After completion, the solvent was evaporated under vacuum at 50 °C. The residue was dissolved in 96% ethanol at 55 °C and filtered off insoluble part. The filtrate was collected and crystalized overnight at 1-5 °C to get crude mesna. The spectroscopic data analysis of mesna structure are given in Table 2.

Table 2. IR, MS, ^{1}H-NMR, ^{13}C-NMR data analysis of mesna

Structure	
IR (KBr): $\bar{\upsilon}$, cm^{-1}	2970 and 2938 (C-H); 2571 (S-H); 1161 and 1047 (SO_3^-)
ESI-MS (MeOH): *m/z*	141.34 [M-Na]$^-$; ($C_2H_5NaO_3S_2$, M = 164.17)
^{1}H-NMR (MeOD, 500 MHz): δ, ppm	2.748-2.779 (2H, m, H-2); 3.064-3.095 (2H, m, H-1)
^{13}C-NMR (MeOD, 125 MHz): δ, ppm	18.37 (C-2); 54.34(C-1)

In previous study, the effects of factors (temperature: reflux (~ 80 °C), volume ratio of water and ethanol = 7:5, volume of solvent: 450 mL ethanol) for preparation of sodium 2-chloroethanesulfonated was investigated[9]. Thus, in this work, the conditions on synthesis of mesna was evaluated.

First, we studied the reaction of sodium 2-chloroethanesulfonate with sodium trithiocarbonate at the different mole ratio (Table 3).

Table 3. The effect of mole equivalence to the yield of mesna synthesis

No.	Amount of Na_2CS_3 (mole)	Mole ratio of 2 : Na_2CS_3	Amount of mesna (g)	Yied (%)	mp. (°C)
1	0.12	1:1	5.75	29.16	264.5-267.0
2	**0.18**	**1:1.5**	**9.98**	**50.61**	**263.5-266.5**
3	0.24	1:2	7.31	37.07	264.0-267.0
4	0.36	1:3	5.03	25.51	263.5-267.0
5	0.48	1:4	4.91	24.89	263.5-267.0

At the mole equivalence between sodium 2-chloroethanesulfoante and sodium trithiocarbonate to 1:1, the yield of reaction was 29.16%. When increased the mole ratio of this mixture to 1:1.5, the reaction generated up to 50.61% yield of product. However, the equivalence of sodium trithiocarbonate was increased continuously, the yield of reaction was decrease. For this reason, we choose 1:1.5 as optimal mole equivalences of two above reagents to carry out next survey with reaction temparature (Table 4).

Table 4. The effect of reaction temperature to the yield of mesna synthesis

No.	Temperature (˚C)	Amount of mesna (g)	Yield (%)	mp. (˚C)
1	50	8.57	43.46	263.5-266.5
2	55	9.96	50.51	264.0-267.0
3	60	9.95	50.45	264.0-267.0
4	70	9.89	50.15	263.5-267.0

With surveyed temperature range (temperature ranging from 50 °C to 70 °C), the yield reached the highest value of 50.51 % at 55 °C. The yield did not change significantly when increasing temperature. However, at high temperature, trace of dimesna which was a side product was seen on TLC. From these results, the temperature of 55 °C was excellent selected for this reaction.

Based on these explorations, optimal condition was chosen to investigate the repeatability of the process with 10 gram per batch in scale (Table 5).

The results showed that mesna was synthesized in a high sustainable yield, about of 50.30 %. Mesna crude samples had melting point in the range of 263.5-267.0 °C. However, content of these samples was 91%, which was lower than that of BP 2015. Hence, a procedure of purification is necessary.

Table 5. The repeatability of the mesna synthetic process

No.	Amount of Na_2SO_3 (g)	Amount of mesna (g)	Yield (%)	Content (%)	mp. (˚C)
1	19	9.85	49.95	90.4	264.0-267.0
2	19	9.96	50.51	91.2	263.5-267.0
3	19	9.95	50.45	91.4	264.5-267.0
Average	**19**	**9.92**	**50.30**	**91.00**	

3.2 Purification of crude mesna

Crude mesna (10.00 g) was dissolved in 96% ethanol (150 mL) at 55 °C, accompanied with supplying continuously nitrogen gas. The mixture was filtered at hot condition to remove insoluble part, filtrate was collected and let to crystallize overnight. Mesna crystals were separated by filtration and refined once again by the same procedure to obtain white solid. The product was dried under vacuum and inert gases at 40°C (Table 6).

Table 6. The repeatability of the procedure for mesna purification.

No.	Amount of crude mesna (g)	Amount of purified mesna (g)	Yield (%)	Content (%)	mp (°C)
1	10.00	9.0	90	98.3	266.0-267.0
2	10.00	8.9	89	98.1	265.5-267.0
3	10.00	8.9	89	98.0	266.0-267.0
Average	**10.00**	**8.93**	**89.30**	**98.2**	

The average yield of purification process reached to be 89.33 %. Product after purification had relative strict melting range and met the content standard of BP 2015. From these results, we conducted to synthesize mesna on 100-gram-per-batch scale.

3.3 Scale-up synthesis of mesna to 100 g per batch

Preparation sodium 2-chloroethane-sulfonate: was carried out by the described procedure with following conditions: using a 10 L flask, 475 mL (6.00 mole) of 1,2-dichloroethane, 251.0 g (2.00 mole) of sodium sulfite, 1875 mL of 96% ethanol, 2625 mL of water, 10.56 g (0.17 mole) of copper powder.

Preparation mesna: was carried out by the described procedure with following conditions: sodium 2-chloroethanesulfonate (whole amount obtained in previous step), 610 mL (2.14 mole) of 40% solution of sodium trithiocarbonate, temperature 55 °C**.**

Purification: by the described procedure with following conditions: using 96% ethanol (in ratio: 1.0 g crude mesna per 15 ml 96% ethanol, hot at 55 ^{o}C) with supplying continuously nitrogen gases, then solution was filtered, crystalized overnight at 0-5 ^{o}C. Mesna crystals were isolated by filtration and refined once again by the same procedure to obtain white solid. The product was dried under vacuum and inert gases at 40^{o}C (Table 7).

Table 7. Results of synthesis of mesna on scale of 100 gram per batch

Amount of Na_2SO_3 (g)	Amount of 2 (g)	Amount of crude mesna (g)	Amount of purified mesna (g)	Content (%)	Yield (%)	mp. (°C)
251	275.38	142.91	127.53	98.6	38.99	266.0- 267.0
251	275.43	142.25	126.75	98.8	38.76	265.5- 267.0
251	274.51	141.60	126.89	98.7	38.80	265.5- 267.0
251	**275.11**	**142.25**	**127.06**	**98.70**	**38.85**	

From 475 mL of 1,2-dichloroethane and 251.0 g of Na_2SO_3, 127.06 g of mesna was obtained in a batch with a total yield of 38.85% as white crystal with mp. 266.0-267.0 ^{o}C. TLC: R_f = 0.33 (*n*-butanol : acetic acid : water = 9.0 : 2.0 : 2.5, *v/v*).

3.3 Evaluation of product quality

The product quality was evaluated by all required standards of BP 2015. The results are shown in the table 8.

Table 8. Testing results about product quality

Norms and applied standards		Requires	Results
1	Characters	White or slightly yellow, crystalline powder, hygroscopic. Freely soluble in water, slightly soluble in ethanol (96 percent), practically insoluble in cyclohexane.	Complies
2	Qualitative analysis *(IR method)*	The IR spectra of the specimen match the reference spectrum of mesna.	Complies
	Sodium *(Chemical method)*	It gives reaction (a) of sodium cation	Complies
3	Appearance of solution	Meet the requirements	Complies
4	pH	4.5 to 6.0	Complies (4.5)
5	Loss on drying	Not more than 1.0%	Complies (0.8%)
6	Chlorides	Not more than 250 ppm	Complies
7	Sulfates	Not more than 300 ppm	Complies
8	Disodium edetate	Not more than 500 ppm	Complies
9	Heavy metal	Not more than 10 ppm	Complies
10	Other relative impurities *HPLC method*		
	Impurities of C	Not more than 0.2 %	Complies (Not detected)
	Impurities of D	Not more than 3.0%	Complies (Not detected)
	Impurities of A, B and E	Not more than 0.3%	Complies (% impurity A, % impurity B=0.001%)
	Other impurities	Not more than 0.1%	Complies (0.0 %)
	Sum of other impurities	Not more than 0.3%	Complies (0.0 %)
11	Assay *(Titration method)*	It contains NLT 96.0% and NMT 102.0% of $C_2H_5NaO_3S_2$, calculated on the dried substance	Complies (98.8%)

4. DISCUSSION

Structure of mesna is relative simple including two functional groups: thiol (-SH) and sulfonate (-SO_3Na) which bond together by ethylene spacer (-CH_2-CH_2-). According to many studies, the synthesis of mesna often includes two main tactics, namely, building -SO_3Na group and making off -SH group. In the reported literature, -SO_3Na group is almost built by Strecker reaction from 1,2- halogenoethane and Na_2SO_3, while -SH group is formed by hydrolysis or reduction of various functional groups, such as thioester, thiouronium, alkyl xanthate or Bunte salt (Figure 3)[6, 7, 9, 12].

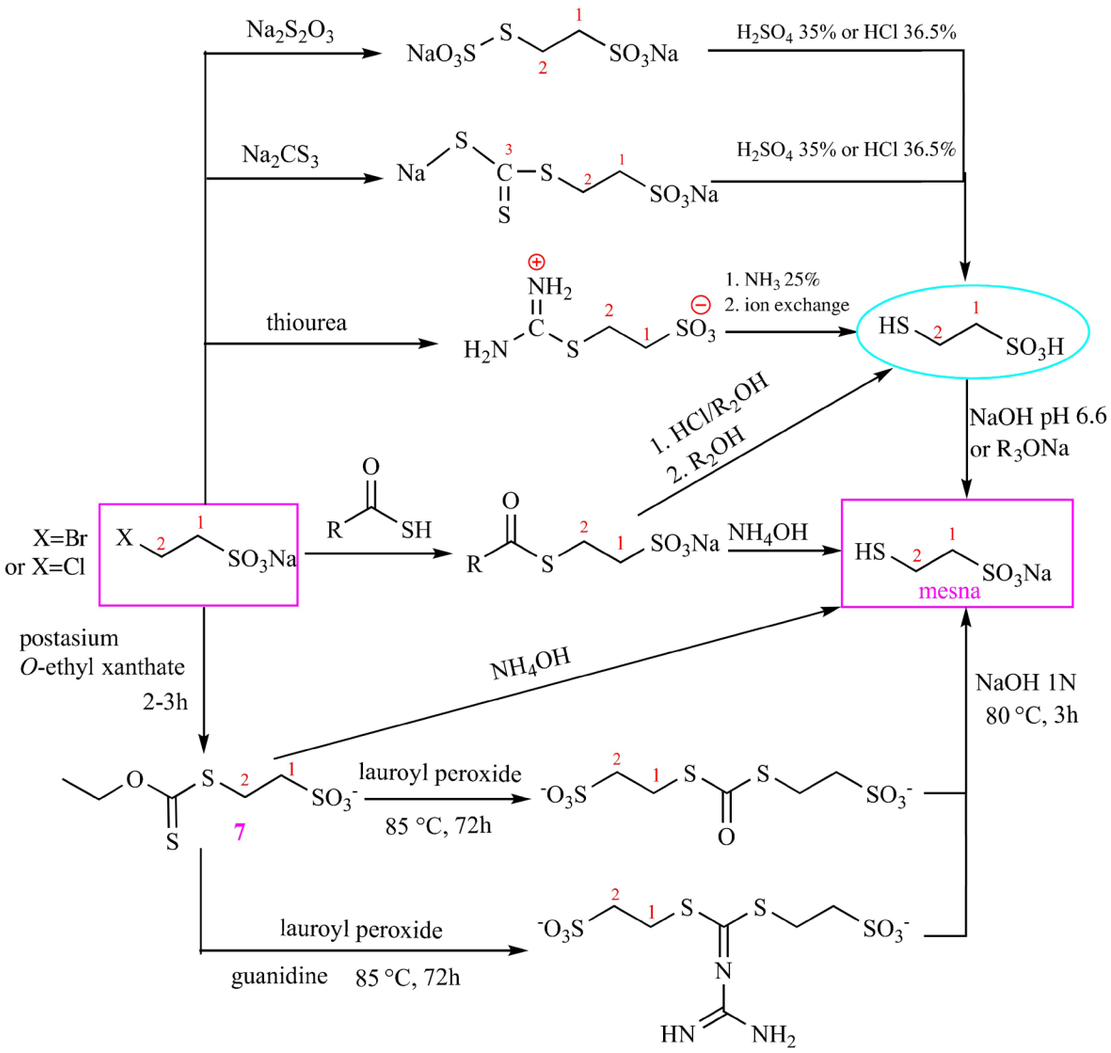


Figure 3. Scheme of various pathways to synthesis of mesna

In this study, we have used Strecker reaction to joint -SO_3Na to ethylene linker, which is similar to procedure of R. Bai[12]. Sodium 2-chloroethanesulfonate has been obtained in a average stable yield of 80% which is comparative with reported yields[9, 12].

To design SH group, in previous work[9], we have found that the hydrolysis of alkyl trithiocarbonate may be applied to afford SH in mesna. This is a relatively simple pathway with the wide accessibility of starting materials, herein sodium trithiocarbonate is prepared from sodium sulfide and carbon disulfide in water by the convenient synthesis procedure[5].

The alkyl trithiocarbonate intermediate synthesis mechanism can be represented in Figure 4.

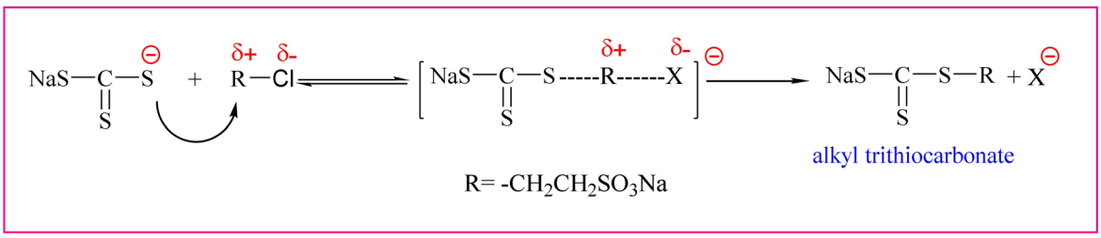


Figure 4. Mechanism of formation of alkyl trithiocarbonate

This process has been described in several references which focuses on the preparation of alkyl trithiocarbonates, aryl trithiocarbonates, and cyclic trithiocarbonates[13, 14]. The trithiocarbonate intermediate synthesized by S_N2 which can produce monoalkyl trithiocarbonate or dialkyl trithiocarbonate derivatives depending on the mole ratios of the reactants (Figure 5).

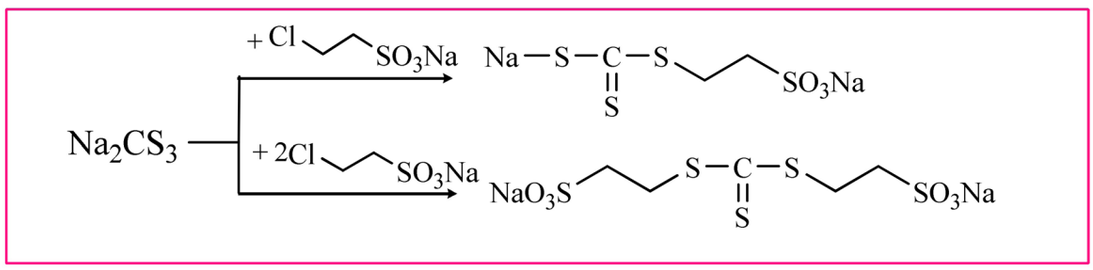


Figure 5. Formation of mono- and di-alkyl trithiocarbonates

According to the references[14, 15], there are many different opinions to obtain thiol from mono- and di-alkyl trithiocarbonates. The reaction may occur by the hydrolytic or reductive mechanism. Particularly, this can be explained as follows: in acidic medium, monoalkyl trithiocarbonate intermediates is transformed in to the unstable compound, which leads to form CS_2 and the corresponding thiol (Figure 6)[13].

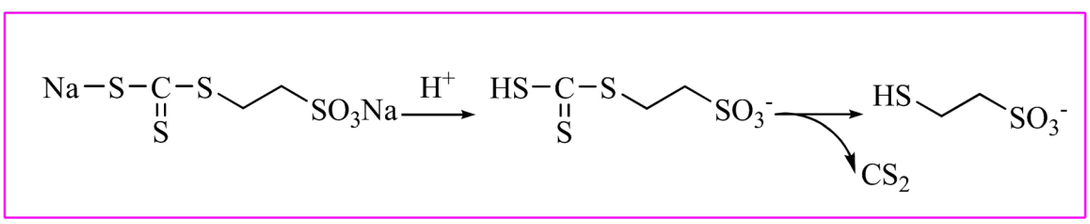


Figure 6. Mechanism of mesna formation

In fact, we have synthesized the intermediate dialkyl trithiocarbonate. However, its hydrolysis did not produce mesna in our experiments. Therefore, mole exploration was important and necessary to afford only mono-alkyl trithiocarbonate, lead to obtain mesna.

Furthermore, we have isolated successfully mono-alkyl trithiocarbonate and demonstrated

that mesna was hydrolyzed from it lead to 37.75 % in yield of whole process[9]. The synthetic *one-pot* process without isolation of mono intermediate has lead the overall yield up to 38.85%. The old condition gives 37.75% of the total yield, that was the first study without any exploration[9]. Current optimization experiments were designed to evaluate effects of the key in-put factors (temperature, mole ratio) to confirm the obtained results. The exploration has showed no significant difference between old and optimized conditions.

Because the mesna group contains the -SH group which is susceptible to oxidation to dimesna, the refining is an important step to obtain pharmaceutical product. We have refined mesna according to the published methods by Jary J et al[5]. After neutralizing to pH 6.6, the solution is concentrated to 1/5 volume at reduced pressure and diluted with 20 times the amount of absolute ethanol. The formed fine crystals are filtered off and dried under inert condition (nitrogen gas). According to the method of Reiner A et al[16], the solution obtained after adjusting the pH is concentrated to dryness, added methanol with ratio 1:15 (theoretical mass of mesna/volume of methanol), and heated to not greater than 40 °C[16]. However, our tries have shown that the final mesna has still many by-products (the traces appear on the thin layer chromatograms). Some other solvents were investigated for the purpose of separating the contaminants from the mesna. We have found that the use of 96% ethanol at a temperature of about 50-55 °C is best condition to purify mesna.

In comparison with the previous reports, this method has some advantages, such as a simple synthetic procedure which included only three steps, a reaction condition easy to control, and a short reaction time. Moreover, starting raw materials of this method are commercially available and be able to supply at larger size. The yield of entire process reached 38.85% which is equivalent to that in synthesizing mesna through intermediate guanidinium 2-mercaptoethanesulfonate[8, 9].

5. CONCLUSION

We have successfully expanded the synthesis of mesna from 1,2-dichloroethane in total yield of 38.85% using alkyl trithiocarbonate approach. The structures of the compounds were defined by IR, MS and NMR spectra data analysis. The product has met all quality requirements of British Pharmacopoeia 2015 therein the content of dried substance was 98.8%. This method can be a practical way to synthesize mesna in the pilot scale.

6. ACKNOWLEDGEMENT

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