College of Basic Education Researchers Journal. ISSN: 7452-1992 *Vol. (19), No.(1), (2023)*

Spectrophotometric Determination of Sulphacetamide Sodium Using 2,4-Dinitrophenylhydrazine as Coupling Reagent

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(Received In 16/11/2022 Accepted In 24/1/2023)

Abstract

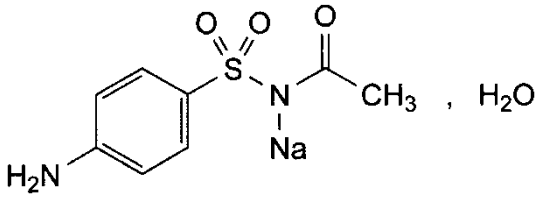
A Sensitive, rapid and selective spectrophotometric method was developed for the determination of sulfacetamide sodium in an aqueous solution. The method is based on the oxidative coupling reaction with 2,4-dinitophenylhydrazine in the basic medium in the presence of potassium periodate to produce an intense orange colour product, soluble in water, stable and gives maximum absorbance at 484 nm. The linearity of Beer's law was in the range of 2.5-75 µg/ml of sulfacetamide. The molar absorptivity, Sandell's sensitivity, and detection limit were 3202.92 L/mol.cm, 0.07936 µg/cm2 and 0.50476 µg/ml respectively. This method is applied successfully to the determination of sulfacetamide in pharmaceutical preparation (eye drops and ointment).

Keywords : Sulphacetamide sodium , oxidative coupling reaction, 2,4- dinitrophenylhydrazine, spectrophotometry.

1.Introduction

Sulfonamides, commonly known as sulfa drugs are benzenoidamino compounds derived from the parent molecule sulfanilic acid that acts as bacteriostatics by inhibiting the formation of dihydrofolic acid, which is essential for the development of bacterial cells [1]. Sulfonamides are an important class of antibacterial drugs used in medicine and veterinary practice [2]. Sulfa drugs are widely used in the treatment of infection sespecially for patients intolerant to antibiotics the vast commercial success of these medicinal agents and a branch of commercial importance in the pharmaceutical sciences[3].Sulfa are widely used in medicine because of their inhibitory effect on the growth in many bacteria [4].They are widely used in the treatment of urinary tract, acne vulgaris,bacterial infection of eye, conjnnctivitis, bacterial infection of skin, seborrhoeic dermatitis , seborrhoieic sicca, trachoma,and nocardiosis, toxoplasmosis[5]. Sulfacetamide (SAA) is a synthetic broad -spectrum antibiotic of the sulfanilamide family[6]. Sulfacetamide is a sulfonamide antibiotic

that is used as a cream to treat skin infections and as eye drops to treat eye infections. On the skin it is used to treat acne and seborrheic dermatitis [7]. And it is used in the treatment of urinary tract infection and burn therapy [8]. It is used for treatment of ophthalmic infections as it is not irritating to eye in high doses [9]. SAS is white,crystalline powder freely soluble in water slightly soluble in ethanol, sparingly soluble in acetone and partially soluble in ether and chloroform[10]. The scientific name for the sulphacetamide is: N-[(4 – aminophenyl)sulfonyl]acetamidemonosodium salt monohydrate and the molecular structure is ($C_8H_9N_2NaO_3S,H_2O$) [11], and its chemical structure as following[12] :



M.Wt = 254.2 g/mol

Several methods have been reported for the determination of sulphacetamide using different techneques such as spectrophotometric methods[13-15], chromatographic methods[16-17], also electrochemical methods[18-19] .The aim of this work is to development of a simple spectrophotometric method for the determination of sulphacetamide sodium in its pharmaceutical preparations .

2.Experimental

Apparatus

All absorbance measurements were done by using a double beam UV-Visible spectrophotometer (JASCO V- 630) with 1.0 cm glass cells. Professional benchtop pH meter TRANS BP3001 was used for the pH measurements.

3.Chemical reagents

All chemicals used in this study were of analytical reagent grade.

SAS solution (500 µg.ml^{-1}) : This solution was prepared by dissolving 0.05 g of pure SAS in enough amount of distell water, then complete the volume to 100 ml by distelled water using volumetric flask and keep dark flask.

2,4-dinitrophenylhydrazine reagent solution(2×10^{-3} M) : This solution was prepared by dissolving 0.0396 g of 2,4-dinitrophenylhydrazine in 5.0 ml of sulphuric acid (6.0 M) and the volume was completed to 100 ml in a volumetric flask with distilled water.

Potassium periodate solution(0.01 M) : This solution was prepared by dissolving 0.2300 g of potassium periodate in distilled water with gentle heating and stirring, and the volume was completed to 100 ml in a volumetric flask with distilled water.

Sodium hydroxide solution (1.0 M) : This solution was prepared by diluting one ampoule of sodium hydroxide solution (10 M) with distilled water, to one liter using volumetric flask.

Excipients solution (10 µg/ml) : This solutions were prepared for prednisolone acetate (as a medicinal substance) and benzalkonium chloride (as a preservative) by dissolving 0.01 g. of each pure substance in a sufficient amount of distilled water, then complete the volume to 100 ml with distilled water, then dilute 10 ml of this solution with distilled water to 100 ml using volumetric flask.

Pharmaceutical solution for eye drops (500 µg / ml) : This solution was prepared by withdrawing 1 ml of the eye drops(Apisulfa-10/Amman Pharmaceutical Industries Co. Jordan) (10%, each 1 ml contains 0.1 g of SAS) and diluted to 100 ml by distilled water , then taking 50 ml of the later solution and diluted to 100 ml with distilled water .

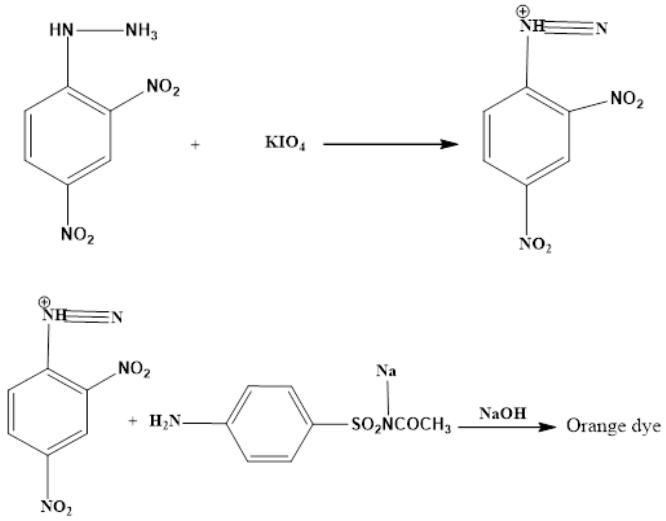
Ointment solution (500 µg/ml): This solution was prepared by weighting 0.5 gm of the eye ointment (Predmacin 10% /Linda-a-vetha ,Portugal) (each 1 g contains 0.1 g of SAS) and then dissolving it in 10 ml of ether and quantitatively transferred to separating funnel followed by addition of 40 ml of ether, and start extraction process by three batches of 25 ml distilled water then collect the aqueous layers containing SAS and transfer it to a volumetric flask of 100 ml capacity, then completing the volume with distilled water to the mark.

4.Results and discussion

To select optimal condtions for SAS determination, deffirent effects were investigated using 1.0 ml of SAS solution (500 µg) in a final volum 20 ml and the absorbance of the solutions was measured against blank solution.

The general principle of the proposed method

The principle of the prposed method depends on two steps the first one : reaction of reagent 2,4-dinitrophenylhydrazine with the oxidant potassium periodate to produce diazonium ion which coupled in seconed step with sulphacetamide in an alkaline medium to form an orange product that has highest absorption at the wavelength of 484 nm. as in the following equations:



Optimum Reaction Condations

The effect studied of different conditions on the absorption of the formed colored product by reaction of 1.0 ml of sulfacetamide (500 µg) with the reagent 2,4-dinitrophenylhydrazine in the presence of potassium periodate in an alkaline medium in a final volume of 20 ml as following:

Effect of oxidizing agent amount

The effect of oxidant amount on the absorption of the colored product formed was investigated by adding different volumes of potassium periodate (0.5-2.5 ml) . The results are shown in the table (1) indicate that addition of 1.0 ml .of oxidant gives the best value of absorbance , so it select for subsecquent expriments .

Table 1 : Effect of the amount oxiding agent

ml of KIO_4(0.01 M)	**0.5**	**1.0**	**1.5**	**2**	**2.5**
Absorbance	0.2664	0.3251	0.2472	0.2212	0.1747

Effect of the amount of coupling reagent

The effect of the amount of coupling reagent on the color intensity was studied by adding different volumes(0.5-2.5) ml of reagent solution (2×10^{-3} M) to the volumetric flask containing 1.0 ml of sulphacetamide (500 µg) and 1.0 ml of oxiding agent (0.01 M), then addition of 2.0 ml of sodium hydroxide and complete volume with distilled water to mark (table 2).

Table 2 : Effect of the amount coupling reagent

ml of 2,4-DNPH (2×10^{-3} M)	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**
Absorbance	0.0999	0.1444	0.2473	0.3277	0.2256

The results in Table (2) show that addition of 2.0 ml of the reagent was optimum amount for higher absorbance for the colored product ,therefore it was adoped for next expriments .

Effect of the amount of sodium hydroxide

The effect the amount of sodium hydroxide (1.0 M) was studied by adding different amounts to the reaction medium volumetric flask containing 1.0 ml of sulphachamide(500 µg/ml) and 1.0 ml oxiding agent of 2,4-dinitrophenylhydrazine reagent 2.0 ml the volume was completed to 20 ml with distilled water and the results shown in Table (3), reveal that addition of 3.0 ml of NaOH solution gives the convinent addition to get higher absorbance of colored product .

Table 3 : Effect of the amount of sodium hydroxide

ml of NaOH 1.0 M	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**
Absorbance	0.0555	0.0987	0.1793	0.2455	0.2516	0.3254	0.2407

Effect the order of additions

The effect of order of additiosn of reaction copmponent on the absorption of the colored dye was studied and the results showns in table (4), indicate that order (II) was best subsequent for reaction components ,so it was chosen for next expriments.

Table 4 : Effect the order of additions

Order number	Order of addition	Absorbance
I	SAS + 2,4-DNPH + KIO_4 + OH^-	0.2681
II	SAS + KIO_4 + 2,4-DNPH + OH^-	0.3238
III	2,4-DNPH + KIO_4 + SAS + OH-	0.2582
IV	SAS + OH^- + KIO_4 + 2,4-DNPH	0.0151
V	2,4-DNPH + OH^- + KIO_4 + SAS	0.009

Effect of temperature

The effect of temperature on the formation of the resulting dye was studied at three different temperatures and the results are shown in the Table (5), confirn that when the reaction is carried out at 40•C ,the absorbance was somewhat higher than it done at R.T , but we chose the room temperature because it moor convinent for labrotary work.

Table 5 : Effect of temperature

Temperature C^0	٠	R.T (25±1)	40
Absorbance	٠,١١٨٤	٠,٣٢٢٧	٠,٣٣٩٨

stability of colored product

The stability of the reaction was investigated at laboratory temperature by measuring the absorbance of the colored product at the wavelength 484 nm. after diluting the solution with distilled water to the mark against its the blank. The results are shown in the table (6) clear the colored product was stable at least for 45 min.which consider as enough period time for carring out several mesuerments .

Table 6 : Effect of color product stability time

Time (min.)	Absorbance / 500 µg SAS
immediatily	0.3167
5	0.3168
10	0.3168
15	0.3164
20	0.3169
25	0.3165
30	0.3167
35	0.3183
40	0.3186
45	0.3198

Effect of adding surfactants

To study the effect of surfactants on the absorption of the formed dye the effect of adding four types of positive CPC and CTAB, negative SDS and neutral Triton X-100 surfactants was studied by adding 3 ml of each type of surfactant after adding all the solutions under optimal conditions and according to the approved method. The results are listed in the table(7) shown that there is no improvement on the absobance of the colrored product ,so it was excluded in the next experiments .

Table 7 : Effect of adding surfactants

3ml of surfactant solution added	λ_{max}	Absorbance
SDS (1×10^{-3} M)	484	0.2998
Triton-100 1%	496	0.2942
CPC (1×10^{-3} M)	499	0.2272
CTAB (1×10^{-3} M)	493	0.2496
Without surfactant	484	0.3242

Effect of the organic solvents

The effect of completing the solutions with organic solvents on the absorbance of formed colored product was studied by dilution with different organic solvents instead of water the results shown in Table (8)and Figure (1) reveal that using n-propanol as solvent gave the higher absobance of colored product , but water still the best solvent due to its safty in use ,also it is lower cast comparring with other organic solvent.

Table 8 : Effect of the solvents

Solvent used	Absorbance	(nm)λ_{max}
Water	0.3232	484
Ethanol	Turbide	Turbide
Methanol	Turbid	Turbid
Acetone	0.2749	503
n-propanol	0.3493	503

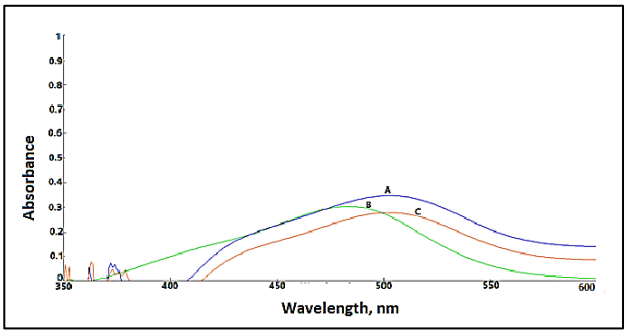


Figure (1): Absorption spectra of the dye formed in the presence of solvents (A) propanol (B) distilled water (C) acetone.

Final absorption spectrum

When adding 1.0 ml of a solution of sulphacetamide sodium of 500 µg/ml to a volumetric flask of a capacity of 20 ml, adding 1.0 ml of the oxidizing agent solution of 0.01 M potassium periodate,

then 2 ml of the reagent solution 2,4-dinitrophenylhydrazine (2×10^{-3}M) and 3.0 mL of 1.0 M sodium hydroxide solution and completing the volume with distilled water to the mark, an orange-colored dye is formed which exhibts maximum absorbance at 484 nm against the blank solution. Figure (2) shows the final absorption spectrum for 500 µg of SAS / 20 ml.

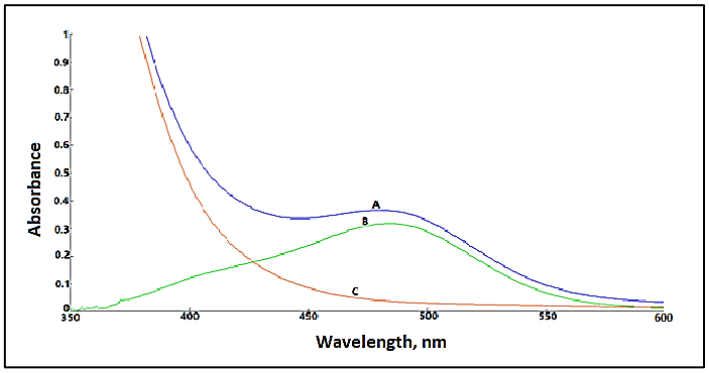


Figure (2) Final absorption spectrum of 500 µg/mL sulphacetamide: (A) sample vs. distilled water (B) sample vs. blank (C) blank vs. distilled water.

Approved working method and calibration curve

After fixing the optimal conditions for the determination of sulfacetamide, the calibration curve for was prepared as follows:

Increasing volumes of standard sulphacetamide solution (0.05-4.0) are added to a series of 20 mL volumetric flask, then 1.0 ml of 0.01 M potassium periodate solution and 2 ml of 2,4-dinitrophenylhydrazine reagent solution(2×10^{-3} M) are added followed by addition of 3.0 mL of 1.0 M sodium hydroxide solution, then complete the volume with distilled water to the mark and measure the absorption against the blank at 484 nm. Figure (3) represents the standard curve for the determination of sulphacetamide, which is consistent with Beer's law in the range of concentrations 2.5-75 µg/ml of sulphacetamide and there is a negative deviation at concentrations higher than 75 µg/ml.The molar absorptivity value is 3202.92 l/mol.cm and Sandell's sensitivity value is 0.07936 µg/cm^2.

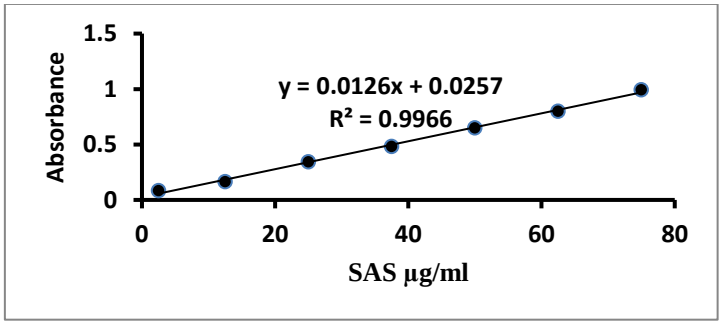


Figure (3) Standard curve for the determination of sulphacetamide according to the approved mode of action.

The nature of the dye formed

To find out the interaction ratio between sulphacetamide and 2,4-DNPH reagent, the Job's method [20]was applied and the following is the method of work:

A number of solutions containing increasing volumes of sulphacetamide 0.1-0.9 ml and corresponding volumes of 0.9-0.1 ml of 2,4-dinitrophenylhydrazine reagent at a concentration of (1.966×10^{-3}) M each of them were prepared, and the rest of the solutions were added under optimal conditions and according to the approved method. Completing the volume with distilled water up to the mark and then measuring the absorption of these solutions against their blank at the wavelength of 484 nm. Figure (4) shows the correlation ratio between sulphacetamide and the reagent 2,4-DNPH is 1:1

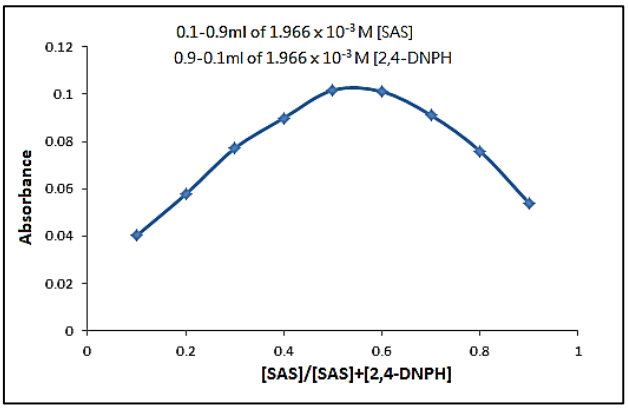
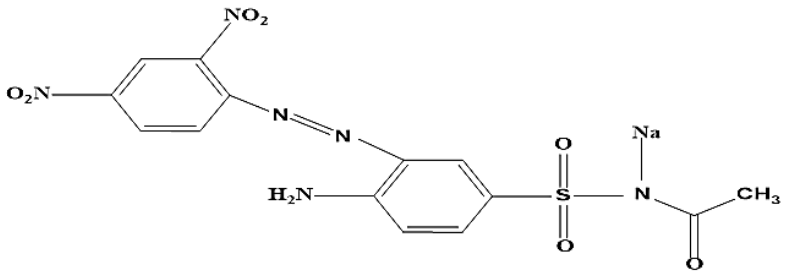


Figure (4) Job's method curve for the dye resulting from the reaction of SAS with the reagent 2,4-DNPH.

From the results of Figures (4) the proposed formula for the formed dye is as follows:



Orange dye

Application of the method

The suggested method was successfully applied to assay the drug in commercial preparation (eye drop and ointment), the results in table (٩) illustrated that the proposed method is accurate and reproducible.

Table 9 : The result of applying of proposed method to pharmaceutical preparations

Pharmaceutical Preparations	*SAS Present* (µg)	*SAS Found* (µg)	Recovery (%)*	Relative error (%)*	RSD (%)*
Apisulfa-10 sterile eye-drops Amman Pharma.Indust.Co.Ltd(Jordan)	500	49.985	98.12	-1.88	1.38
	1000	97.640	97.41	-2.59	0.38
Predmacin 10% ointment Linda-a-vetha (Portugal)	500	49.215	103.47	3.47	2.81
	1000	99.11	102.24	2.24	0.26

*Average of five determinations

The results in Table (9) indicate the success of the proposed method for the determination of sulphacetamide in its pharmaceutical preparations.

Evaluate the result using the standard addition method

For the purpose of proving the efficiency and accuracy of the proposed method for estimating sulphacetamide in pharmaceutical preparations, the standard addition method was applied to two different concentrations of the medicinal preparation. The results are shown in Figure (5) and Table (10).

Table (10) : Recovery results for SAS in its pharmaceutical preparations by proposed method and standard addition method

Pharmaceatical Preparation	Amount taken, µg	Recovery %	
		Present method	Standard method
Apisulfa-10 sterile eye-drops Amman Pharma.Indust.Co.Ltd(Jordan)	٢٥٠	98.12	٩٨,٤٠
	٥٠٠	٩٧,٤١	٩٩,٢٠
Predmacin 10% ointment Linda-a-vetha (Portugal)	250	١٠٣,٤٧	٩٩,٦٠
	500	١٠٢,٢٤	٩٩,١٦

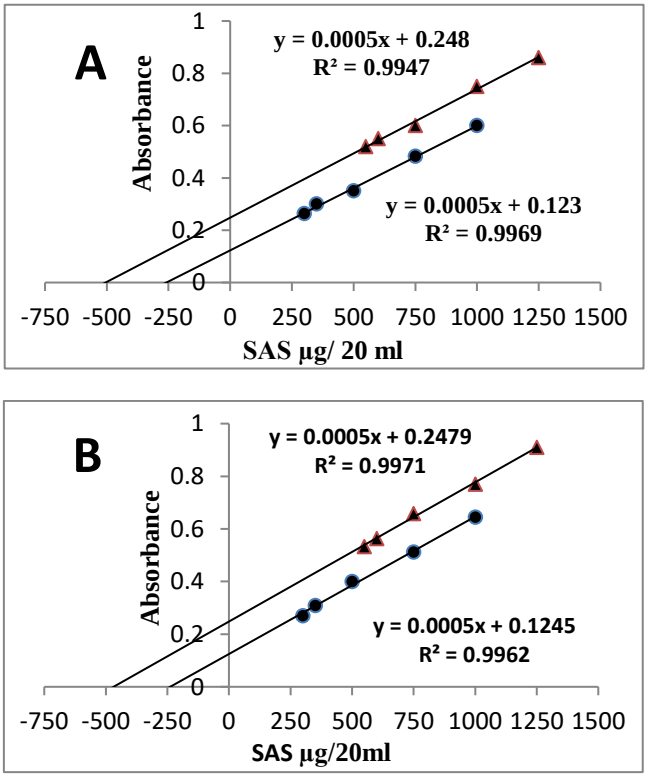


Figure (5) Standard addition curves for the determination of sulfacetamide in pharmaceutical preparations (A) eye drop (B) ointment.

The results in the figure (5) and table(10) revealed that the recoveries of SAS in standard addition method is in good agreement with the proposed method, which indicates a good selectivity of the proposed method .

5.Comparison of the method

Some of the analytical spectroscopic variables and application of the proposed method for estimation of sulfacetamide sodium were compared with the same variables for other spectroscopic methods . Results shown in table (١1) reveal that the proposed method is analytically significant from point of view, where it has wide range of determination and acceptable sensitivity .

Table (11): comparison some spectroanalytical of the variables proposed with other spectrophotometric method for the determination of sulphacetamide sodium.

Analytical parameters	Present method	Literature method[21]
Reaction types	Oxidative coupling	Oxidative coupling
Reagents	2,4-dinitrophenylhydrazine	Phenothiazine
λ_{max}(nm)	484	610
Medium of reaction	Basic	Acidic
Color of the product	Orange	Green
Beer's law range (ppm)	2.5-75.0	0.1-16
Molar absorptivity (L.mol^{-1}.cm)	3202.92	2.599×10^4
Sandell sensitivity (µg/cm^2)	0.07936	0.0101
L.O.D (µg/ml)	0. 50476	/
Applied pharmaceuticals	Eye drops & Ointment	Eye drop only

6.Conclusions

An easy and quick spectrophotometric method has been developed for the determination of sulphacetamide in pharmaceutical preparations. The method involves oxidative coupling reaction of sulphacetamide with 2,4-dinitrophenylhydrazine in the presence of potassium periodate in an alkaline medium to form a stable, water-soluble orange dye, which gave the highest absorption at 484 nm. The method has been successfully applied to deteremination of sulphacetamide in its pharmaceutical preparetions as eye dropos and oiment .

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