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Development of a 'drop-count' method for the screening of the ethanol content in hand sanitisers using oxalic acid and potassium permanganate titrimetry

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ABSTRACT

Introduction

The demand for ethanol-based sanitisers has increased dramatically since the dawn of the COVID-19 era, making it very difficult for health regulators to ensure the quality of the products and the safety of unsuspecting consumers. This article reports the development and application of a simple 'drop-count' titrimetry method for the determination of ethanol in hand sanitisers.

Materials and Methods

This method follows the classical potassium permanganate-oxalic acid titrimetry but uses very small volumes of the reagents – 0.5 mL of ethanol sample solution, 6 mL of acidified 0.1 M potassium permanganate, and a few counted drops of 0.1M oxalic acid.

Results

This method achieved satisfactory figures of merit, namely, linearity **(R2 ≥ 0.9667)**, reproducibility, and importantly very little matrix effect in the alcohol concentration range of 50 to 80%.

Conclusion

The small volumes (< 10 mL in total) of the reagents required make this method considerably safe and cost-effective and applicable for fieldwork even by untrained personnel, not just chemists as is often the case for field applications.

INTRODUCTION

The emergence of the COVID-19 pandemic has generated extensive socio-economic and health challenges around the globe. As expected from any mega-event, the COVID-19 pandemic generated hype of activity worldwide. Voluminous information on this pandemic – informative and/or misleading, became available on many media platforms. Given the status of the pharmaceuticals regarding the development of vaccines, the skepticism and outright disinformation/conspiracy theories about the pandemic and the vaccines (Romer & Jamieson, 2020), other non-pharmaceutical measures are taking a centre stage as it is very little to no conspiracy theory in their use (Perra, 2021), and importantly they are mostly cheaper alternatives.

The non-pharmaceutical approaches approved by the World Health Organisation (WHO) to manage the pandemic include wearing face masks to prevent the spread of infections, regularly washing hands with soap and running water, frequent disinfection of surfaces and hands, keeping distance between individuals, and isolation or quarantine of the infected. Wearing face masks has been the major facet of the fight, followed by the use of hand sanitisers that have proven to be the best-opted disinfectors as regular washing of hands with running water would be highly impractical for people on the move. This has increased the market and demand for these commodities with a possibility of luring unscrupulous producers to enter the market, putting pressure on regulatory bodies.

Hand sanitisers are antiseptics that fall into the low-risk Class 1 - they have no risk to human health as long as they are used per their designation category (Kurnia, 2020). However, with the ease with which these sanitisers can be produced, coupled with the increased demand, many opportunistic producers can cash in on the rush by producing sub-standard sanitisers (with less alcohol percentage) to meet the high demand. This has made health officials deployed in the fight against the pandemic question the quality of most sanitisers. Some studies have reported the presence of some questionable products that do not meet the required standards in Italian Markets (Berardi et al, 2020), while in Johannesburg, the economic capital of Southern Africa, 37 of the 94 samples (41%) collected from formal retail stores, informal convenience shops, and by street vendors, contained less than 60% alcohol (Matatiele et al., 2022).

The compliance should not only be restricted to the minimum requirements for effectiveness but should also include the quality of the product. Some studies indicate that some products do not meet other quality standards and that could cause harm to unsuspecting consumers (Cohen et al, 2021). Hence the need for the health regulators to carry out chemical screening of commercial sanitisers to ensure that producers are legitimate and that sanitisers produced are up to standard and are produced as per the regulations and guidelines provided by WHO - at least 60% ethanol (U.S. Food and Drug Administration, 2020).

There are several methods used to quantify the alcoholic content in products to ensure the quality of the highest standard and most are based on chemical reactions (titration), physical properties (e.g. pycnometry), and instrument-based - most of which have been summarised recently (Sriariyanun, 2019). Different techniques including spectroscopy - infrared (Fonseca et al, 2020), Raman (Cleverland et al, 2007) as well as electrochemistry (Paixão et al, 2002) offer alternative methods to the commonly used chromatographic methods (Meden, 2016). However, these techniques come with a considerable cost to laboratories with resource limitations.

Despite the availability of many instrumental methods as already alluded to, simple wet chemistry methods such as oxidation by potassium dichromate and by potassium permanganate remain the only viable option for most economic regions as they are not only easier to carry out, but most importantly they are cost-effective as they do not require the use of expensive equipment. However, these methods rely on colour change as the ethanol-containing substance is titrated with an oxidising agent which is a challenge for coloured products. Other challenges include the fairly large amounts of reagent solutions required, leading to large quantities of waste and contaminated glassware which would need to be carefully rinsed to enable further use subsequently and to remove hazardous chemical residues, especially in the case of dichromate (Science ASSIST Team, 2020).

As the demand for field operations increases, chemists have developed simple methods for screening ethanol in sanitisers. Recently, a colorimetric screening method that estimates the amount of ethanol in liquid samples has been reported (Langhals, 2020). This article reports the

development of a simple, cheap, and rapid method for the estimation of the ethanol content in sanitisers, based on direct 'drop-count' titrimetry and its application in assessing the sanitisers available in the market for compliance concerning ethanol content. This method is based on the titration of alcohol using the number of drops (drop count) of the oxalic acid in potassium permanganate. The method further simplifies the analysis in that it eliminates the need for volumetric calculations of the molarities before converting the moles to percentage composition, consequently it can be used even by untrained personnel, not only chemists. A related report on a miniaturised technique was made by Nogueira et al (2019) where they immobilised the required reagents on a piece of paper. However, this method employs 3-D printed material and it is more complicated than the reported approach.

MATERIALS AND METHODS

Reagents and apparatus

All the reagents, namely, potassium permanganate, oxalic acid, hydrogen peroxide, and ethanol (99.8%) were of analytical grade and obtained from Merck® (Johannesburg, South Africa). Concentrated sulphuric acid was obtained from Laboratory Analytical (Durban, South Africa). Glycerol was obtained from a local store in Roma, Lesotho.

Preparation of oxalic acid and potassium permanganate

Approximately 0.3 M solutions of oxalic acid and potassium permanganate were prepared in 250 mL volumetric flasks, respectively. The potassium permanganate was acidified by mixing it with 3 M sulphuric acid and prepared and from this, an aliquot was obtained and added to a standardised solution of 0.3 M of potassium permanganate in one volumetric flask. This was done in volume ratios of 2:1 for H_2SO_4 and $KMnO_4$, respectively.

Preparation of samples and titration

Volumetric titration of samples of ethanol only

Different standard solutions of ethanol (50%, 60%, 70%, and 80%) were prepared by diluting the 99.8% stock ethanol. To 0.5 mL aliquots of each ethanol sample in a 100 mL Erlenmeyer flask, 75 mL of acidified 0.1 M $KMnO_4$ was added and shaken. The reaction mixture was then heated to about 70°C in a hot-water bath. Thereafter, this mixture was titrated to a clear (discolouration) endpoint with 0.1 M oxalic acid. The titrations were carried out in triplicate and the average volume of oxalic acid used for each sample was recorded. Then a plot of volume versus ethanol concentration was drawn.

Drop-count titration of samples with a different ethanol content

To 0.5 mL aliquots of each of the ethanol samples (50%, 60%, 70%, and 80%) in a test tube, 6 mL of acidified $KMnO_4$ was added and shaken. The reaction mixture was then heated in a hot-water bath set at about 70°C, thereafter titrated drop-wisely while counting the number of drops of oxalic acid until a clear solution was obtained. For each sample, three trials were performed and the number of drops of oxalic acid used for each sample was recorded. Thereafter, a plot of the number of drops of oxalic acid versus ethanol content was drawn to be used as a calibration curve.

The same procedure was followed to determine the effect of (i) glycerol - 1.45%, (ii) hydrogen peroxide - 0.125%, and the (iii) WHO recommended formulation (1.45% glycerol and 0.125% hydrogen peroxide) in the ethanol solutions.

Method validation

Samples of ethanol, glycerol, and hydrogen peroxide were prepared blindly and for each sample, 0.5 mL was reacted with 6 mL acidified $KMnO_4$ in a test tube. The reaction mixtures were then heated to about 70°C in a hot-water bath and immediately drop-wisely titrated with oxalic acid until a clear solution was obtained. For each sample, three trials were performed and the number of drops of oxalic acid used for each sample was recorded.

RESULTS AND DISCUSSION

Development of the method

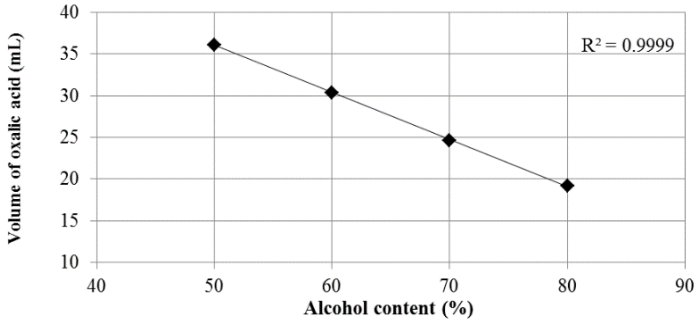
Standardisation of the potassium permanganate with oxalic acid

Standardisation is very important in chemical analysis. However, given that the approach does not require the usual volumetric calculations, it was not necessary to show the results of standardisation; it is safe to state that the calculated molarity of potassium permanganate was 0.31 M.

Volumetric titration of ethanol standard solutions

Since this method is dependent on volumetric titration, it was considered prudent to calibrate the volumes so that no molar calculations can be employed in the field. Hence it was important to determine the linearity of the correlation between the volumes of the oxalic acid required to consume the remaining permanganate after reacting with ethanol in the sample (see **Figure 1**).

Figure 1:
Correlation of volume of oxalic acid against the percentage of alcohol in the sample. The error bars (too small to see) represent the standard deviation of the volume for n=3.



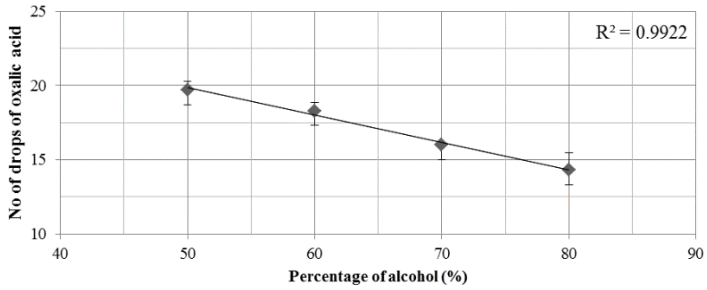
The results show that as the amount of alcohol in the sample increases the volume of the oxalic acid used in titration decreases. This is because more of the potassium permanganate was involved in the redox reaction with ethanol and less of it was involved in a back titration reaction with oxalic acid. The results show sufficient linearity **(R2 = 0.9999)**, demonstrating that for ethanol concentrations between 50 and 100% there is a sufficient correlation between volumes of oxalic acid used in back titration against the ethanol content.

Drop-count titration of ethanol with oxalic acid

Given that the method is intended for field operations, it is important to use very small volumes of all the reagents used. Hence, the volumes of the ethanol-containing sample, the permanganate, and oxalic acid, all have to be reduced by the same ratio. A drop-wise titration was carried out using Pasteur pipettes instead of burettes to transfer the oxalic acid into the ethanol-permanganate mixture. Unlike burettes, Pasteur pipettes are portable, simple, and cheap, making the method more suitable for fieldwork. The end-point of the titration was reached when the brown solution from the ethanol and potassium permanganate reaction became colourless. **Figure 2** shows the number of 0.1 M oxalic acid drops as a function of ethanol content in the ethanol aqueous standard while titrating 2.0 mL of 0.1 M permanganate.

As can be seen from **Figure 2**, the drop-based method gives comparable results to the normal volumetric titration except for slightly lower linearity (**R2 = 0.9922** compared to **0.9999**). This indicates that the drop-count method can be used for field operations and is safe for lower precision.

Figure 2:
The plot of the number of drops of oxalic acid versus the percentage of alcohol in the aqueous samples. The error bars represent the standard deviation of the volume for n=3.



Assessment of the matrix effect from the WHO prescribed preparation protocol

According to the WHO standards, a sanitiser must have 0.125% hydrogen peroxide, 1.45% glycerol, and about 70% of alcohol. Given that glycerol increases the amount of the titratable OH groups, it is important to estimate the contribution of the glycerol to the drop-count titration. Equally important is the addition of hydrogen peroxide, which can react with the permanganate (see Reaction Scheme 1), leading to the increased ethanol erroneously. To assess the effect of glycerol and hydrogen peroxide, different solutions of ethanol were prepared at different concentrations and glycerol was added to one series of solutions and hydrogen peroxide added in another series. The solutions were thereafter titrated as the other ethanol solutions:

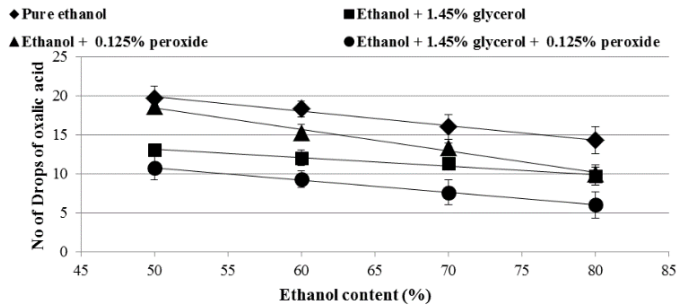
$$5H_2O_2 + 3H_2SO_4 + 2KMnO_4 \rightarrow 5O_2 + 8H_2O + 2MnSO_4 + K_2SO_4 \quad (1)$$

Figure 3 shows the correlation of the number of drops of oxalic acid versus alcohol content in different matrices according to the WHO prescription i.e. pure ethanol solution, ethanol, and 1.45% glycerol, ethanol, and 0.125% hydrogen peroxide and the mixture of the three reagents – ethanol + glycerol + hydrogen peroxide combined in one figure for ease of reference.

When reacted with $KMnO_4$, hydrogen peroxide reduces the total amount of $KMnO_4$ available to react with the ethanol thus dropping the number of drops of oxalic acid. Similarly, glycerol reacts with potassium permanganate consequently reducing the potassium permanganate available for alcohol reaction. This increase in the amount of potassium permanganate that reacts thereby increases the amount of

alcohol present in the sample because the calculations are based only on the back titration of the excess potassium permanganate. This means that samples with both hydrogen peroxide and glycerol will appear to have a higher alcohol percentage than expected. Therefore, the sample (sanitisers) labels must state whether or not the samples contain both chemicals, and at how much, so that a correct estimate can be made confidently.

Figure 3:
Drop-count titration of alcohol in different matrices with oxalic acid



For a simpler comparison, the number of drops with and without hydrogen peroxide as well as those with the glycerol content are presented in **Table 1**.

Table 1:
Comparison of the average number of drops of oxalic acid in different ethanol concentrations and different matrices

Matrix	80	70	60	50	Linearity, R^2
Ethanol (simple aqueous solution)	14.3	16.0	18.3	19.7	0.9922
Ethanol + 1.45% glycerol	9.67	11.33	12.0	13.0	0.9667
Ethanol + 0.125% H_2O_2	10.0	13.3	15.3	18.6	0.9996
Ethanol + 1.45% glycerol+0.125% H_2O_2	6.0	7.6	9.3	10.7	0.9887

Application of the method to real samples

Comparison of the drop-count method with the volumetric titration using sanitiser samples prepared in the lab

Since only the drop-count titration will be applied for fieldwork, it is crucial to first apply both normal titration and drop-count titration on the same sanitiser samples to see if comparable results can be obtained. **Table 2** shows the results of the determination of the samples using the volumetric and drop-count approaches.

The average and standard deviation calculated for n=5, the two decimal places were necessary to allow for some degree of variance which would not be observed for one decimal place derived from the 0.5mL volume of the sample used.

Table 2:
Comparison of the two methods using 70% and 60% ethanol laboratory samples

Analyses	Drop-count method		Volumetric method	
Stated	70.00	60.00	70.00	60.00
Obtained average*	71.05	60.00	69.99	59.99
Standard deviation*	3.61	3.15	0.96	0.64

From **Table 2**, the volumetric method seems to be more precise as the standard deviations for the two samples are much less than those from the drop-count method. This is understandable given that a drop-count method relies heavily on the counting of the drops irrespective of the size of each drop.

Determination of ethanol content in laboratory sanitiser samples prepared according to WHO guidelines

Three samples of laboratory sanitisers with 65, 70, and 75% were prepared and tested accordingly resulting in 64.8±4.7%, 70.4±4.2%, and 73.6±5.4% respectively which indicates that the method is considerably precise with a standard deviation of less 5.4%. In fact, for all the determinations for the samples prepared in the lab, the 5.4% was the highest recorded relative standard deviation, thus indicating that the method is quite reproducible for the intended application. Without carrying out any statistical t-tests, it can be observed that the obtained values are sufficiently close to those stated on the containers indicating satisfactory accuracy for a screening technique.

Application of the drop-count method to commercial samples

The basis of drop-count titrimetry is ordinary titration and the results obtained for the two methods have shown some correlation in the experiments conducted above (see **Figure1 & 2**). It is therefore important to check if the drop-wise titration can be directly used to determine the alcohol percentage in commercial sanitisers so that it can be applied for fieldwork. The method was therefore applied to four different commercial sanitisers and yielded positive results as shown in **Table 3**.

Table 3:
Determination of alcohol percentage in commercial sanitisers

Samples	Determined (%)	Claimed (%)
Gel sample	76.5 ± 0.0	75
Liquid sample A	73.5 ± 3.7	70
Liquid sample B	72.0 ± 4.2	70
Liquid sample C	72.5 ± 2.7	> 60

Given that the commercial samples did not have any certificate of analysis, no further accuracy validation could

be explored. It must be mentioned that some commercial samples that were obtained for analyses in our laboratory failed the tests. However, they were not part of this study so their results were not included in this report.

CONCLUSIONS

This drop-count method was successfully developed based on performing redox titrations and determining the alcohol content in hand-sanitiser samples. This method offered good analytical performance; a satisfactorily linear correlation **(R2=0.9922)** between alcohol content and the number of drops of oxalic acid was achieved within the percentage range of 80 – 50% of alcohol content. Importantly, considering the low sample consumption (0.5 mL) used for analysis, within a short time, the method is quite efficient and cost-effective. Needless to emphasise, this method is instrumental-free. Despite the applicability of the method, the glaring effect of glycerol and hydrogen peroxide suggests that the sanitiser labels should clearly state whether or not they contain both chemicals, and how much so that a correct estimate can be made confidently.

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Ethical Approval: Nil

Conflicts of Interest: None declared.

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