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Chemical stability of heparin, isopropanol, and ethanol line lock solutions

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ABSTRACT

Background: Ethanol line locks are used in the US to prevent catheter associated bloodstream infections. Heparin precipitates in solution with ethanol. However, isopropanol may reduce precipitate formation. We aimed to determine the chemical stability of heparin, isopropanol, and ethanol line lock for a 10 day period at 2–8 °C and 25 °C.
Methods: Forty samples were prepared for analysis. Each sample was prepared identically using a 5 ml syringe capped with a Combi-stopper: 1 ml 70% isopropanol, 1 ml 70% ethanol, and 1 ml heparin sodium 10 IU/ml. Twenty syringes were stored at 2–8 °C and 20 at 25 °C. Analysis was carried out on days 1, 3, 6, 8, and 10 with a single syringe from each condition being tested in duplicate. Samples were assessed visually. Sub-visible particle count analysis was carried out using a CLIMET particle counting system. Heparin concentration was analysed using an anti-Xa assay. Ethanol and isopropanol concentrations were analysed by gas chromatography.
Results: Samples remained clear and colourless throughout the study. Sub-visible particle counts remained within limits specified in British Pharmacopoeia 2013 when stored at 2–8 °C and 25 °C, 60% humidity for up to 10 days. There was no significant change in ethanol or isopropanol concentration during the study. However, heparin activity fell by >10% after 1 day storage and to 65% of original activity after 10 days.
Conclusions: This study shows that addition of isopropanol to heparin and ethanol prevents precipitation. However, this solution shows a progressive decline in heparin activity over time making it unsuitable for extended shelf life.

1. Background

Central venous catheters (CVCs) provide reliable venous access for haemodialysis, blood sampling, monitoring, plasmaphoresis and the administration of fluids, drugs, chemotherapy and parenteral nutrition. Catheter related blood stream infections (CRBSIs) are associated with a significant morbidity and mortality for the individual leading to an increase in health care costs estimated to be £9148 per CABSI [1]. In 2000, the National Audit Office reported that hospital-acquired infections were each year costing the NHS around £1000 million and resulting in at least 5000 deaths [2]. Standardising the insertion and maintenance of central lines by introduction of care bundles has reduced CRBSI rates [3]. Line-locks are a routine part of care bundles used to prevent CRBSIs and maintain intraluminal patency during intervals when the CVC is not being used [4]. Different line-locks are used including saline, heparin, ethylenediaminetetraacetic acid (EDTA), citrate, ethanol, taurolidine and antibiotics [5].

Ethanol line-locks are widely used in the USA as they are cheap and widely available. Ethanol has shown to have great potential to eradicate organisms in biofilms and to treat or prevent CRBSI [6]. The American Pediatric Surgical Association has recommended ethanol as a safe and effective line-lock in the prevention of CRBSI (grade A/B recommendations). The mechanism of action is by denaturing bacterial proteins and may be particularly effective in colonised CVCs where organisms are protected by a biofilm. It does not induce bacterial resistance and exhibits broad spectrum activity including gram negative organisms and fungi [7–9] with no known resistance [10]. Two recent reviews evaluating the evidence supporting ethanol line locks concluded that ethanol is effective in preventing CRBSI but conclude that occlusive events appear to be a concern [10,11]. Several studies have reported problems with precipitation and CVC occlusion using ethanol locks particularly with totally implantable devices [12–14]. A recent *in vitro* study assessing precipitation in heparin/ethanol solutions concluded that concentrations of ethanol (>28%) exhibited significant precipitate and suggested that this may account for reported vascular access device occlusion events [6].

The addition of an anticoagulant to an ethanol lock may prevent CVC occlusion. Similar approaches have been used with antibiotic locks [15]. Traditionally heparin has been considered incompatible with ethanol because it forms a precipitate [16]. The drug monographs of numerous manufacturers of heparin and ethanol line locks state that ethanol and heparin precipitate in combination. An intriguing *in vitro* study reported that the solubility of heparin is greatly enhanced by judiciously combining isopropanol and ethanol instead of using ethanol alone [17]. Isopropanol has similar pharmacokinetic and toxicology profiles to ethanol [18].This abstract is the only such report in the literature and does not correlate the effect of incubation time on precipitation. The clinical

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application of this study hinges on whether the heparin, isopropanol and ethanol solution is stable in storage, the toxicity profile of isopropanol and whether the biological activity of the heparin and ethanol is preserved.

Our aim was therefore to perform an *in vitro* study designed to determine the chemical and physical stability of heparin, isopropanol and ethanol line lock solution in syringes for a period of up to 10 days at both 2–8 °C and at 25 °C, equivalent to refrigerator and room temperatures, respectively.

2. Methods

2.1. Materials

Table 1 lists the sources of materials used. Stock 70% isopropanol solution was made using sterile water for irrigation as a diluent.

2.1.1. Sample preparation and storage

Study samples were prepared at Quality Control North West Liverpool. Preparations were carried out in such a way so as to eliminate any particulate contamination in samples used for sub-visible particle count analysis. Samples were prepared on different days over a 10 day period, stored at 2–8 °C refrigerated or 25 °C in a 60% RH humidity cabinet, and analysed simultaneously on the final day, such that the properties of solutions at days 0, 1, 3, 6, 8 and 10 could be determined. Prior to any analysis, sample syringes underwent a standard mixing process by drawing 1 ml air, attaching a Combi-stopper, and inverting 10 times.

2.1.2. Samples prepared for sub-visible particle count analysis

On each day of preparation, ampoules of ethanol and heparin solution were opened. Taking a new syringe and needle, 1 ml 70% isopropanol was drawn, followed by 1 ml ethanol 70%, and 1 ml heparin 10 IU/ml. This process was repeated four times, using the same ampoules and stock isopropanol. After preparation, four syringes were split, with two stored at 2–8 °C and two at 25 °C.

2.1.3. Sample prepared for heparin, isopropanol and ethanol assays

On each day of preparation, ampoules of ethanol 70% and heparin solution 10 IU/ml were opened. 4 ml 70% isopropanol, ethanol 70% and heparin was added to a measuring cylinder using calibrated pipettes. The solution was mixed and allowed to stand for 30 min to equilibrate to room temperature, before being divided amongst four syringes. The syringes were split, with two stored at 2–8 °C and two at 25 °C.

2.1.4. Sampling protocol

On the final day, all 40 syringes were removed from storage. 10 sub-visible particle count samples were analysed in-house, 10 were delivered to the coagulation laboratory at the Royal Liverpool and Broadgreen University Hospital for analysis of heparin, and the remaining syringes were used for analysis of ethanol and isopropanol. For the sub-visible particle count analysis, two syringes from each storage condition, for each time point, were sampled from a single syringe. For the heparin analysis, a single syringe from each storage condition, for each time point, was tested in duplicate. For the analysis of ethanol and isopropanol, a single syringe from each storage condition for each time point was tested in duplicate, for each analyte. Two vials of heparin 10 IU/ml, an ampoule of ethanol 70% and stock 70% isopropanol solution were also analysed to provide initial assay figures at time point zero.

Table 1
The sources of materials used.

Material	Source
Hydrogen	VWR International Ltd®
Helium	
Ethanol	
Isopropanol (propan-2-ol)	
Propan-1-ol	
Sterile water for irrigation	Fresenius Kabi Ltd
Heparin sodium flushing solution 10 IU/ml 5 ml ampoules	Wockhardt®
Ethanol 70% 'for catheter flushing' 5 ml ampoules	Tayside Pharmaceuticals
5 ml Luer-Lok® syringes	BD
Combi-stoppers for syringes	Braun
Neolus 0.8 × 40 mm Nr2 Luer syringe needles	Terumo®

2.1.5. Heparin assay

Heparin was analysed using an Anti Xa assay. Plasma was quantitatively added to samples and control standards.

2.1.6. Ethanol and isopropanol analysis

Gas chromatography was used to analyse ethanol and isopropanol using an Agilent 6890 N gas chromatograph. Separation was performed at 35 °C on an HP-5 capillary column (J&W Scientific), and elution was obtained with helium carrier gas (flow rate 1.5 ml/min; sample volume 0.1 μl injected using Split injection mode). The chromatography assay was quantitatively validated in terms of standard repeatability and linearity, and in terms of sample repeatability and recovery.

2.1.7. Sub-visible particle count analysis

A CLIMET particle counting system was employed, set to detect particle sizes >2, 5, 10, 15 and 25 microns. Sterile water was used as a control. This was carried out in triplicate, both at the start of the run and again at the end. Two syringe samples for each time point, from each storage condition, were sampled from. The results from the first syringe were used to equilibrate the system and were discounted. The results from the second syringe were reported only. Counts were performed on 1 ml aliquots of sample and converted to counts per 3 ml contained in each syringe.

2.1.8. Stability calculations and statistical analysis

Shelf life may be defined by the length of time from preparation or manufacture of a drug until its original concentration or potency has been reduced by 10% [19]. One-sample t-tests were therefore performed to determine if the concentration or activity of the analytes during the study (i.e. days 1 through 10) were significantly different, either greater or smaller, than 90%. Further one-sided t-tests were performed to determine if sub-visible particle counts were significantly different from the British Pharmacopoeia [20] limits for injection. The limits for injection supplied in containers of this size are stated as: 6000 at >10 μm and 600 at >25 μm. A P value of 0.05 was accepted at the level of significance.

3. Results

All samples remained clear and colourless throughout the study period.

There was an immediate decrease in heparin activity observed after preparation, falling below 90% by day 1. This trend continued with less than 67% heparin activity remaining after 10 days at 2–8 °C. A similar trend was seen at 25 °C with the lowest recorded activity seen after 8 days with less than 57% heparin activity remaining (Fig. 1). The result at 2–8 °C results shows a potential outlier on day 6. Nevertheless, one-sample t-tests confirm that heparin activity is significantly <90% during the study overall at 2–8 °C ($P = 0.002$; mean 65.5; difference −24.5; 95% CI −37 to −12.) and 25 °C ($P = 0.0002$; mean 69.2; difference −20.765; 95% CI −28 to −13). It can be concluded that shelf life of heparin within the study solution does not exceed 10 days.

The ethanol concentration dropped by less than 1.3% (2–8 °C) and 1.5% (25 °C) compared to the initial assay at time zero (Fig. 2). Throughout the study, ethanol concentrations were significantly higher than 90% at 2–8 °C ($P < 0.0001$; mean 99; difference 9; 95% CI 8.8 to 9.2) and 25 °C ($P < 0.0001$; mean 99: difference 9.1; 95% CI 8.7 to 9.4).