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This work was supervised and directed by Dr Jenny Scott, Lecturer in Pharmacy Practice, University of Bath, UK. The practical work was undertaken by four final year pharmacy students for their Masters of Pharmacy honours project. The students involved were: Yee-Kee Cheung, Caoline Quinn, Joanna Slegg and Michelle Wong. This report is based on the honours thesis of Joanna Slegg.

BACKGROUND

The sharing of injecting paraphernalia is a problem among injecting drug users (IDUs). The sharing of such equipment is associated with the transmission of blood borne viruses (BBVs). To prevent BBV transmission, the supply of single-use injecting paraphernalia is advocated. In France the Steribox is available to IDUs. This is a kit which contains everything that the IDU needs to prepare their drugs for injection. It is intended that by supplying such kits the transmission of BBVs will be reduced.

Under French law, all physicians are authorised to prescribe high dose buprenorphine (Subutex®) as drug maintenance therapy for opiate dependence However, the illegal injection of Subutex ® tablets is a growing problem in France[1]. As Subutex ® is not intended for parenteral administration, the injection of this sublingual tablet formulation can result in the introduction of insoluble particles into the vascular circulation. This can cause medical complications such as granulomas, deep vein thrombosis and phlebitis[2]. In order to reduce the risks of injecting insoluble particles IDUs are encouraged to filter their drug solutions before they are injected. Makeshift filters are used for this purpose, commonly a piece of cigarette filter or a piece of cotton wool. The Steribox contains a filter, however it is unknown how well this filter performs in terms of particle reduction. Additionally, if a purpose-designed filter could be produced which removes particles but does not remove drug or add fibres, this would be preferable from a risk reduction viewpoint.

The purpose of these experiments is to investigate the effectiveness of the current Steribox filter compared to the effectiveness of two new prototype filters made by

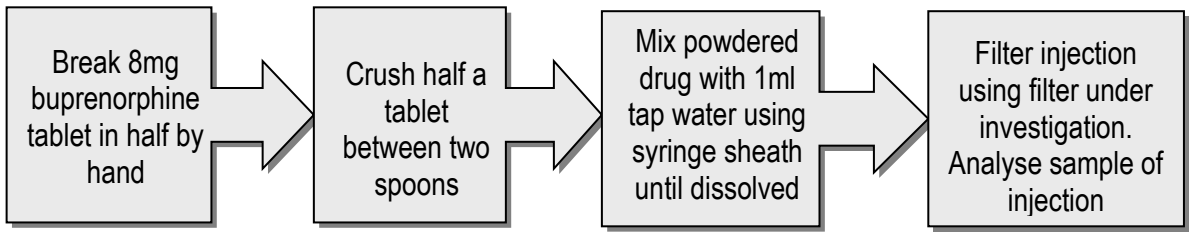
Pall and Millipore . The filters are also compared against makeshift filters known to be used by IDUs for comparison.

It is hoped that the results will be able to inform the development of the contents of the revised Steribox kit.

Methods

Injections were prepared using standard methods developed on the advice of colleagues in France from Association Apothicom. The purpose was to copy what French IDUs do as closely as possible in the laboratory to reproduce conditions experienced in practice. Two preparation processes were developed. One where the injection is prepared on a spoon (denoted sp), the other where the injection is prepared using the Steribox. This allowed comparison with and without Steribox use. The preparation processes are described as follows (fig 1):

i) using spoons



ii) using Steribox

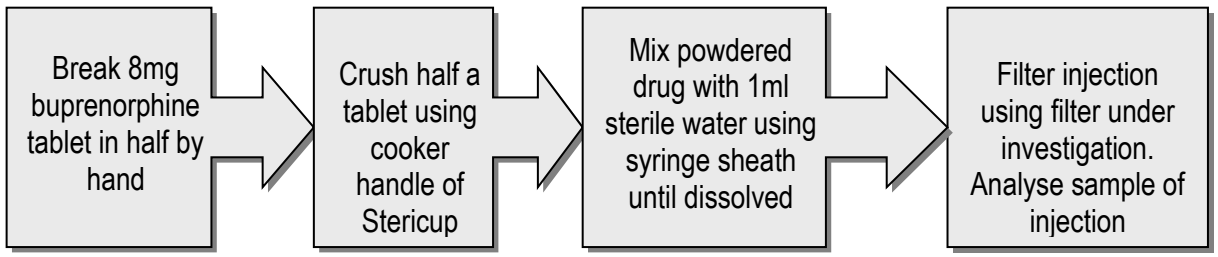


Fig 1: The injection preparation processes used in this study

Once the injections were prepared samples were removed and analysed for particle content using a Coulter Multisizer. The concentration of buprenorphine in the final injections was also measured using High Performance Liquid Chromatography.

Control injections were prepared as above but in the absence of buprenorphine to allow the contribution of the preparation process to particle content to be explored. All methods were validated before use. Experiments were repeated several times, as details below and results were compared statistically.

Materials and Equipment

Filters

Table 1 details the filters that were tested in these experiments and their preparation method.

Table 1: Filters used and their preparation prodecures

Filter name & manufacturer	Material	Preparation	Pore size (µm)
Cigarette (Lambert & Butler, Nottingham, UK)	Cellulose acetate	Paper removed then cut into 5mm pieces	20µm
Rizla extra filter tips, ultra slimline acetate 6mm (Rizla UK Ltd, Mid Glamorgan, UK)	Cellulose acetate	Cut in 5mm pieces	20µm
Cotton bud tip (Unichem, Surrey, UK)	100% cotton	Pulled from plastic stalk and end fibres smoothed	unknown
Dental (Hartmann, Germany)	Pure absorbent cotton wool rolled in thin layers. Outer layer fixed with a neutral bonding agent	Cut into 5mm pieces	unknown-
Steribox 1	Cellulose acetate	Used whole	Unknown
Pall (provided by Association Apothicom, Ivry-sur-Seine, France)	Polypropylene	Used whole	20µm
Millipore (provided by Association Apothicom)	Polypropylene	Used whole	10µm
Acrodisk (Gelman Sciences, Michigan, USA)	Versapor® (acrylic copolymer on a nylon support)	Used whole	5µm

Cigarette filters, Rizlas and cotton bud tips are commonly used by IDUs as makeshift filters. Dental filters are distributed in the UK to IDUs by Barking and Havering Health

Authority as they are thought to be effective at reducing the incidence of medical complications due to particle injection. However, to date no evidence exists for their effectiveness in this context. The term ‘Steribox 1 filter’ has been used to describe the filter that is currently provided to IDUs in France as part of the Steribox injecting kit. Pall and Millipore filters are prototype filters that have been designed specifically for potential inclusion in the Steribox 2. The Acrodisk is a commercial filter.

Particle count analysis materials

A standard Coulter Counter conducting fluid, Isoton II (Azide – free balanced electrolyte solution) was supplied by Beckman Coulter (High Wycombe, Bucks, UK) and VacuCap super-membrane filters (0.2µm) by Gelman Laboratory (Ann Arbor, USA). Latex standards for validation were supplied by Coulter Electronics (Bedfordshire, UK). Buprenorphine hydrochloride sublingual tablets 8mg (Subutex ®) were supplied by Schering-Plough Ltd (Herts, UK).

Concentration analysis

All solvents were of HPLC grade. Acetonitrile and Methanol were supplied by Fisher Chemicals (Loughborough, UK). Orthophosphoric acid and sodium pentanesulphonic acid were supplied by BDH Laboratory Supplies (Poole, UK). Reckitt and Colman (Hull, UK) supplied pure buprenorphine free base. Subutex tablets were supplied as above.

Analysis Conditions & Measurements

Particle count

A Coulter Multisizer II ® (Coulter Electronics, Bedfordshire, UK) was used to count and size the particles in samples (of known volume) taken from buprenorphine injections prepared using different filters. The Multisizer operates on the electrical zone sensing principle. It comprises two electrodes in a beaker of conducting fluid separated by a glass probe with an orifice of 100µm diameter. The conducting fluid, Isoton, was filtered twice using a VacuCap vacuum pump (pore size 0.2µm) to minimise the number of non-sample particles present. A glass stirrer ensures particles remain suspended in the isoton. The Multisizer functions by detecting a change in resistance (measured as a voltage pulse at a constant current) when a particle passes through the orifice. In this investigation, the Multisizer was set to record the total number and size of particles within the size range 2 - 60µm (since the manufacturer guarantees accuracy between 2 and 60% of the orifice size) drawn through the orifice in 12 seconds (the default standard analysis time). Since it could not be guaranteed that the conducting fluid itself was completely particle free, a background count of the number of particles in the beaker of isoton was necessary before each count was performed which could later be subtracted to give the total number of particles in the buprenorphine injection sample alone.

Before the start of each count, the orifice and stirrer were rinsed with isoton to prevent cross-contamination of samples. A 100ml glass beaker was filled with 75ml of isoton and the orifice and stirrer placed in the beaker. The door of the Multisizer was closed to prevent particles from outside the sample contaminating it. The stirrer speed was set to ‘1’ and the isoton allowed to stand for approximately 1 minute to

allow any air bubbles to be eliminated. A background count was performed and the results noted. Following resetting of the machine, 50µl (measured with a Gilson pipette) of the prepared injection sample was added to the beaker containing isoton and the door closed. The sample was left to stand for 30 seconds to allow the injection to mix uniformly with the isoton. A count was performed and the results noted. After each count, the beaker was rinsed with isoton and then refilled with 75ml of clean isoton. Five control injections and five buprenorphine injections were prepared with each filter and particle counted.

The Multisizer produces two measurements: the total particle count and the corrected total particle count. The corrected count is the measurement produced after the machine adjusts for particles that may have been double counted or miscounted and this is the result that was recorded. Additionally, the number of particles within each of four size channels: 1.978 – 4.946, 4.946 – 10.14, 10.14 – 15.08 and 15.08 – 20.03µm were recorded. (The exact size channels were determined by the calibration of the machine and the above were selected as those that were nearest to the ranges 2-5, 5-10, 10-15 and 15-20µm). The size distribution of the particles was recorded so that the data obtained could be related to blood vessel diameter of the microcirculation and the potential risk of vessel blockage of the injection assessed.

Concentration analysis

A Milton Roy liquid chromatograph was used with UV detection to measure the concentration of buprenorphine in sample injections prepared using the eight different filters. The components of a sample are eluted from a column at varying

times (retention time) depending on the extent to which the column retains each component, which in turn depends on its chemical structure. The HPLC method was adapted from a paper published by Tebbett detailing a method of biological assay for buprenorphine by HPLC[3]. The sample preparation stage was modified since the paper is concerned with the analysis of human serum samples, which required specific sample preparation (including centrifugation and alkalisation). The injection samples were prepared as described under 'sample dilution' below. An additional change was made to the published analysis method. An external standard was used in preference to the internal standard. The use of an external standard is more appropriate because the addition of an internal standard such as codeine to the sample would have the potential to introduce error to a system that was already subject to a series of dilutions and therefore this would reduce accuracy. There would also be the danger of the internal standard peak being eluted at a similar time to the buprenorphine peak therefore obscuring it. An external standard of known buprenorphine concentration (10µg/ml) was used and run before and after each set of sample injections and an average of the two peak heights taken.

The mobile phase used was 0.05M sodium pentanesulphonic acid – acetonitrile – methanol (30:15:55) adjusted to pH 2.0 with orthophosphoric acid. The machine was set to deliver the mobile phase at 1ml/min. The samples were monitored at 290nm by an ultraviolet detector. The column used was a 5µm 150mm x 4.6mm Inertsil ODS-3 and the injection loop volume was 20µl. It was expected that when using this method, the retention time for buprenorphine would be approximately 3 – 4 minutes[3].

Before any samples could be run, the buprenorphine concentration range that would fit onto to the integrator print out as well as the exact buprenorphine retention time needed to be established. This was done using samples of known drug concentration and it was concluded that the concentration range easily detectable and measurable was approximately 5 – 70µg buprenorphine/ml and the retention time always fell between 2.96 and 2.99 minutes.

Sample dilution

Each sample injection was diluted by a factor of 400 before analysis by HPLC (fig. 2). This was based on the assumption that each injection would contain half an 8mg buprenorphine tablet (approximately 4 mg) per ml and on the detectable range mentioned above. A sample injection prepared with each filter was analysed three times and an average of the three peak heights calculated. Each sample was allowed to run for five minutes.

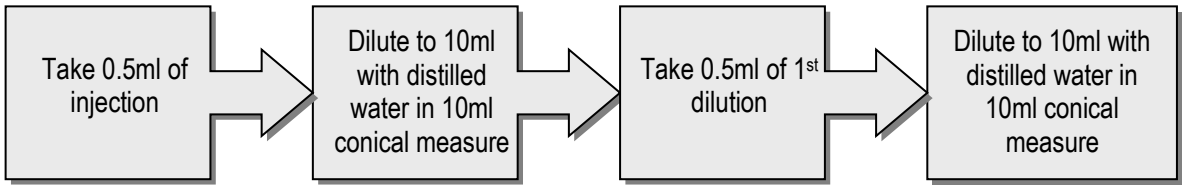


Fig.2. Dilution of sample injections.

It is usual practice to filter any samples for analysis by HPLC with an HPLC filter (pore size 0.2µm) to avoid the introduction of large particles onto the column. Obviously, passing the injection samples through an additional filter would defeat the purpose of the investigation since it would be impossible to separate the effects of the HPLC filter and the tested filters on buprenorphine concentration. Comparison (using the Multisizer) of an unfiltered sample injection and an HPLC filtered injection

showed that there was no significant difference between the number and size of particles present. It was therefore decided that the sample dilution factor of 400 was sufficient to eliminate any large particles with the potential to block the column and filtration with the HPLC filter was not required.

Use of filters

Subjective opinion on the ease and convenience of use of each filter were recorded throughout the course of the investigation.

Table 2 describes how each filter was used during the injection preparation process.

Table 2: Method of use of filters

Filter type	Method of use
Makeshift (cigarette, Rizla, cotton and dental) *	Filter placed at edge of spoon/cooker just touching drug solution. Needle placed on edge of filter not in contact with drug solution and solution drawn up with syringe through filter.
Steribox 1	As for makeshift filters
Prototype (Pall and Millipore)	Filter placed over top of needle and drug solution drawn up with the syringe.
Acrodisk	Needle removed from syringe and replaced with filter. Drug solution drawn up with syringe.

Validation

Particle count

The Coulter Counter was calibrated prior to use and the calibration constant (Kd) was recorded as being equal to 933.08. Before and throughout the use of the machine, the Multisizer's sizing function was validated for accuracy and reproducibility using standards of known latex sizes (10.2 and 5.06µm). By performing counts on the known standards daily, it was found that the machine was

capable of consistently measuring the size of particles accurately. The relative standard deviation (RSD) of measurements recorded on each day was calculated to be between 0 and 1.2%**.** Operator variability was eliminated by using the same researcher each day to perform specific tasks.

Concentration analysis

Before and during the HPLC injection analysis, the machine was validated. Specificity was first looked at to ensure that the response seen (i.e. the peak at approximately 2.96 minutes) was definitely arising from the buprenorphine and could not be a result of any other compound present in the sample. The simplest documented method of validating selectivity is to demonstrate a lack of response to a blank sample. This was done by running a series of 'blank' injections (i.e. made in the same way as the sample injections but without the buprenorphine) and observing for peaks. Since no peaks were seen for the blank injections, this confirmed that the peak at 2.96 minutes was a response to buprenorphine.

If a linear relationship exists between buprenorphine concentration and its peak height, the concentration of buprenorphine in a sample may be calculated by comparing buprenorphine peak height to external standard peak height (as long as the concentration of external standard is known). To determine whether or not there was a linear relationship between the concentration of buprenorphine and its peak height, a calibration curve was required. A straight line calibration graph (fig. 3) was obtained for buprenorphine (R^2 = 0.9803) using pure buprenorphine base based on peak height measurements for concentrations of 5, 10, 20, 30, 40, 50 and 60µg/ml. Each point was calculated as an average of three measurements.

Fig. 3. Calibration graph for buprenorphine standard solutions

R^2 is a measurement of correlation. For analysis where accuracy of results is vital, a higher R^2 than the one above would be desirable. However, for the purpose of this investigation, the R^2 value of 0.9803 was considered sufficient to illustrate a linear relationship between buprenorphine concentration and peak height.

Precision and reproducibility were validated by running three buprenorphine samples of known concentration each day and calculating the RSDs of the peak heights to establish both same day and day to day variation. An RSD of less than 5% is generally considered to be sufficient to illustrate machine precision and reproducibility. For each same day results the RSD was calculated to be under 4% and the RSD of the results measured on different days was less than 3% which confirmed the machine's precision.

Statistical comparisons

Particle size analysis of filtered injections

To determine whether the type of filter used had a statistically significant effect on the number of particles in a buprenorphine injection, a Kruskall-Wallis test was carried out. Dunnett's test was used to compare filtered injections to the unfiltered injection. All injections were then compared to each other using Tukey's test.

Concentration analysis

A Kruskall-Wallis test was performed to establish whether or not a significant difference existed between the buprenorphine concentrations of the injections.

All statistical tests were carried out using the computer programme Minitab. A description of the tests performed is given in table 3.

Table 3: Description of statistical tests employed [a]

Name of test	Use	Measurement produced
[29] Kruskall-Wallis	Compares set of non-parametric data to identify whether or not a significant statistical difference exists (but not where, i.e. between which means)	p-value - the probability that differences in the data has occurred by chance. If $p < 0.05$, there is a significant difference present.
[30] Dunnett's*	All comparisons are made to one control group	Confidence intervals (CI) for the difference between each group and the control. If a CI includes zero there is no significant difference.
[29] Tukey's*	Compares every number to every other number and identifies where significant differences lie.	Confidence intervals for the difference between each pair of means.

* Post-hoc are only required if the Kruskall Wallis test shows that a significant difference ($p < 0.05$) exists in the data.

Results

Particle count

Table 4 is a summary of the results. Averages are calculated from the five individual measurements for each filter except for Millipore (prepared on the spoon) and Pall (using Steribox kit) for which averages are calculated from four results due to one anomalous result for each due to a damaged filter.

Table 4: The effect of filtration on the number of particles in an injection made with half of a Subutex 8mg tablet.

Filter	Ave. no. particles counted in 12 s after filtration (see fig.3)*	Percentage reduction in no. of particles compared to unfiltered (see fig.4)	RSD of five counts (%)
None (spoon)	13418	-	12
None (using Steribox kit)	11974	11	41
Cigarette	10413	22.	15
Rizla	8442	37	41
Cotton	5915	56	72
Steribox 1 (using Steribox kit)	1818	86.	25
Dental	1363	90	22
Steribox 1 (spoon)	1030	92	39
Pall (spoon)	460	97	72
Pall (using Steribox kit)	253	98	38
Millipore (using Steribox kit)	92	99	27
Millipore (spoon)	76	99	37
Acrodisk	17	100	80

RSD = (standard deviation / mean) x 100

**Please note this does not mean the average number of particles in the injection. A standard method was used allowing samples to be compared. This is the number of particles counted in 12 seconds when a 50 microlitre sample of injection is added to 75ml of Isoton analysis fluid.*

To find out whether or not there was a significant difference between the average number of particles per injection for each filter, a Kruskall Wallis test was performed ($p < 0.001$). Since p is less than 0.05, it can be concluded that a significant difference

does exist between the number of particles in each sample. Dunnett's test was then used to compare the number of particles in all the filtered injections to the number of particles in the unfiltered injection prepared using the spoon (the injection with the highest total particle count). A significant difference ($p < 0.05$) was found between the number of particles in the unfiltered injection and all the sample injections except for the injection filtered with the cigarette filter, which was not found to be significantly lower (CI: -7721 – 1713).

Table 4 shows that injections filtered with Acrodisk resulted in the greatest percentage reduction in number of particles compared to the unfiltered injection. However, Tukey's comparison showed that there was no significant difference between the number of particles in the Acrodisk injection and the injections filtered with the Steribox 1 (CI = -5730 - 3704), Pall (CI = -5161 - 4274), Millipore (CI = -4964 - 4471) or the dental (CI = -6063 - 3371) filters. In addition, no significant difference was found to exist between the number of particles in the injections filtered with the cotton, Rizla and cigarette filters. This is indicated in table 4 by the black horzontal line defining the distinction between filters.

The results of Tukey's test were also used to examine the effect on number of particles per injection of using the complete Steribox injecting kit to prepare the buprenorphine injection instead of the spoons. For all injections prepared in both ways (those filtered with Steribox 1, Pall, Millipore and the unfiltered injections) no significant difference was identified.

Table 4 shows the relative standard deviations of the five particle counts for each sample. It can be seen that the RSDs calculated are high and extremely variable and

serve to illustrate that the results obtained are not reproducible, probably due to the nature of sample preparation in which a buprenorphine tablet was broken in half by hand. However, reproducibility of results is not of importance in this investigation since trends in particle counts are being looked at rather than exact particle numbers.

Table 5: Size distribution of particles in injections. Sp = prepared on the spoon. SB = prepared using the Steribox 1

Filter	Percentage of particles within size range			
	1.978 – 4.946µm	4.046 – 10.14µm	10.14 – 15.08µm	15.08 – 20.03µm
None (sp)	30	33	24	11
None (SB)	34	33	22	9
Cigarette	22	41	27	8
Rizla	22	42	27	8
Cotton	24	33	25	8
Dental	43	45	9	1
Steribox 1 (sp)	44	42	10	2
Steribox 1 (SB)	43	46	11	1
Pall (sp)	46	30	16	6
Pall (SB)	62	27	8	3
Millipore (sp)	89	11	2	0
Millipore (SB)	95	21	2	1
Acrodisk	80	1	20	7

Table 5 illustrates the size distribution of the particles in each injection. The Multisizer only counts particles within the size range 2 - 60µm. Therefore the percentage distribution illustrated above does not necessarily add up to 100 since some particles present in the sample may have been less than 2µm or greater than 60µm. In addition, the size channels overlap making it likely that some particles have been detected as present in two channels and as a result counted twice.

Figure 4 shows the proportion of the total number of particles that measured less than 5µm. This is an important measurement as the smallest vessels of the microcirculation (the capillaries) measure 5 - 9µm in diameter. Any particles larger than this may have greater potential to block the vessels and cause the medical

complications described in the introduction. An injection containing as few particles as possible, and of those present as high a proportion of particles as possible less than 5µm in diameter is desirable.

Fig.6. Size distribution of particles

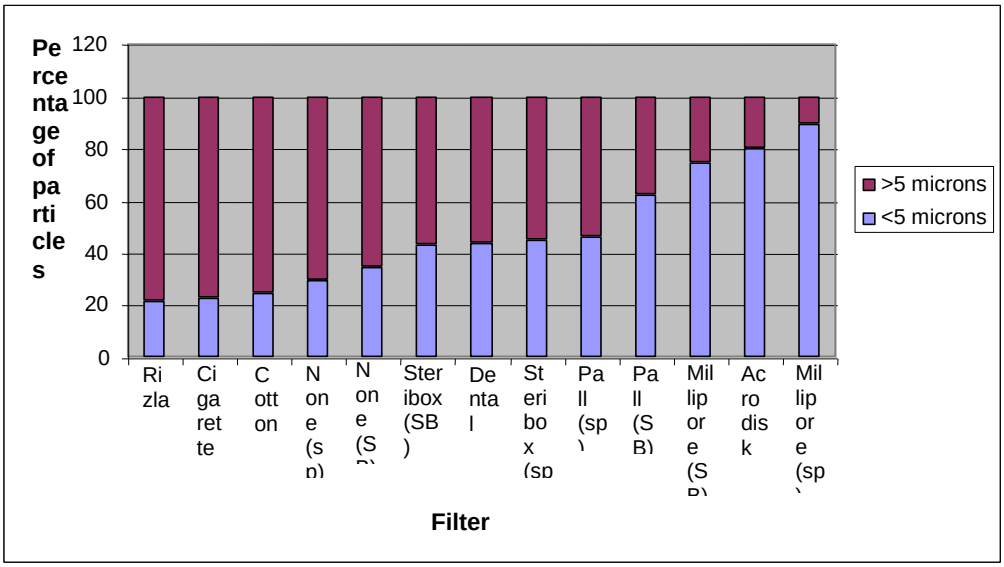


Figure 4 shows that the Millipore filter resulted in the greatest reduction in particle size range with almost 90% of the particles measuring less than 5µm. Of the makeshift filters, the dental filter showed the greatest particle size reduction.

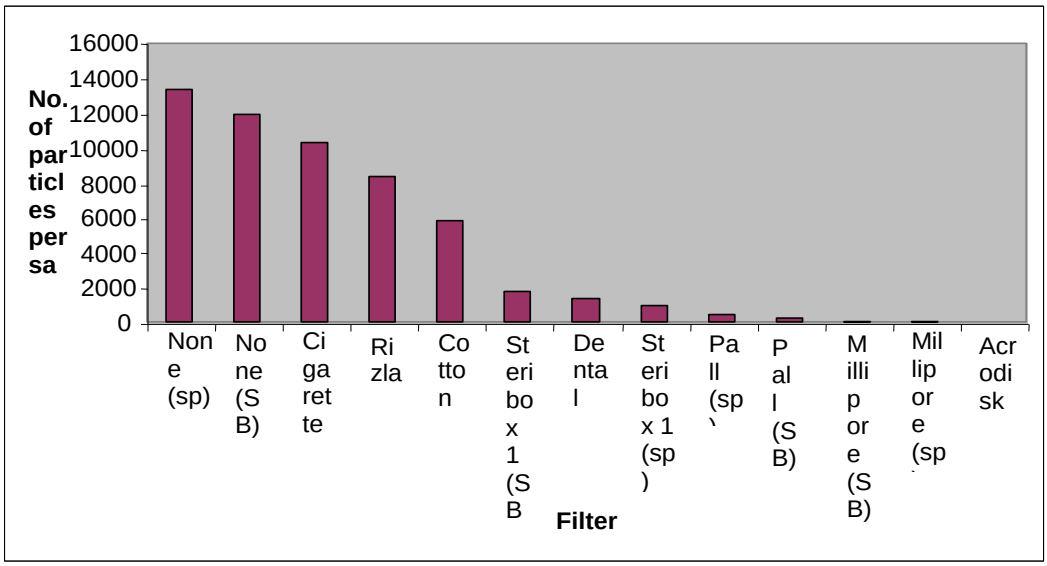


Fig.4. Average number of particles for each filter injection experiment.

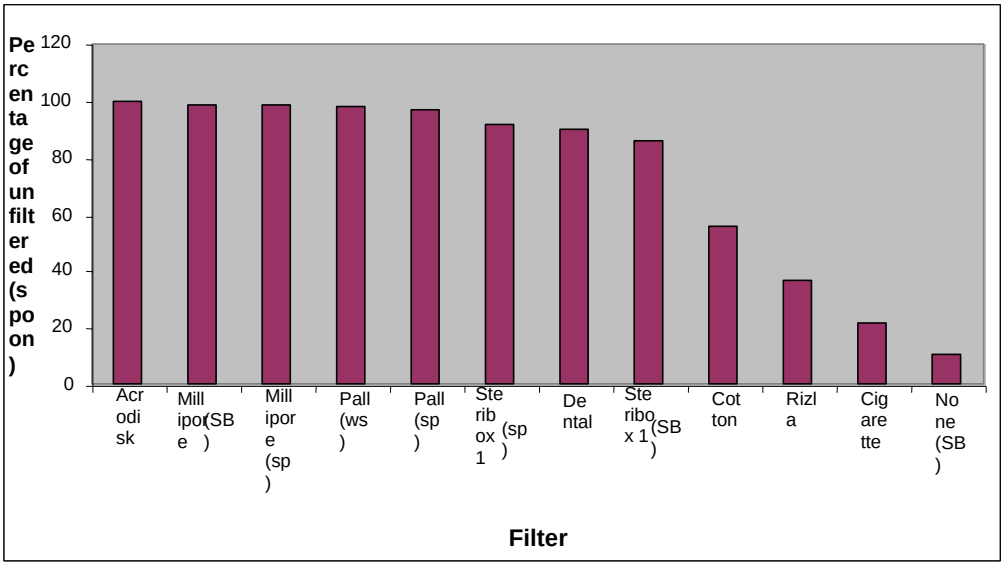


Fig.5 Percentage reduction in number of particles after filtration

Table 6: Contribution of particles by injection preparation

Filter	Average number of particles in control sample	Percentage of control unfiltered injection	Control particle count as percentage of sample particle count
None (sp)	4	-	0.0
None (ws)	20	500	0.2
Cigarette	7	175	0.1
Rizla	8	200	0.1
Cotton	13	325	0.2
Dental	9	205	0.7
Steribox 1 (sp)	5	125	0.5
Steribox 1 (ws)	4	100	0.2
Pall (sp)	24	600	5.3
Pall (ws)	30	750	11.9
Millipore (sp)	24	600	31.1

Millipore (ws)	21	525	22.7
Acrodisk	0	0	0.0

The total particle count of the injection could not be assumed to be entirely a result of the buprenorphine as there were many other factors that could have introduced particles to the injection (see section 4.1). The control injections (i.e. without buprenorphine) were therefore prepared and particle counted in order to assess the contribution of the process of preparing the injection to the total particle count. Table 6 shows the number of particles in filtered control injections compared to the number of particles in the unfiltered control injection. The results indicate that the use of any form of filter (except the Acrodisk) to prepare injections results in the introduction of particles. Comparison of the number of particles in control injections and sample injections prepared with the same filter (table 6) showed that the number of particles introduced by the process of injection preparation (i.e. not by buprenorphine) was insignificant in all cases except for the injections filtered with Pall and Millipore. The most likely explanation for this is that as these filters were very efficient, the total number of particles present in the sample injection was low and thus the number present in the control injection was high in comparison. If this were the case, a high percentage would also be expected for the Acrodisk injection as this had an even higher percentage reduction in number of particles compared to the unfiltered injection than Pall or Millipore. However, the Acrodisk was so efficient that the average number of particles in the control injections was zero, which obviously resulted in a percentage contribution of injection preparation equal to zero.

Concentration analysis

Table 7 shows the final buprenorphine concentration of the samples which were caluculated form the following equation:

$$BN\ (mg/ml) = \frac{\text{Average sample PH}}{\text{Average external std PH}} \times \text{concentration of external std} \times \text{dilution factor}$$

Where, concentration of external standard = 10µ/ml
dilution factor = 400

Table 7
Final buprenorphine concentrations of injections after filtering

Filter	Average sample PH	Average external standard PH	Concentration of buprenorphine in injection (mg/ml)
Unfiltered	31.17	35.00	3.56
Millipore	32.50	32.50	4.00
Steribox 1	31.33	32.25	3.88
Acrodisk	25.00	33.25	3.01
Cotton	32.83	34.00	3.86
Pall	34.00	35.00	3.88
Cigarette	34.50	34.50	4.00
Rizla	33.17	35.00	3.79
Dental	31.83	35.00	3.64

N.B. PH = peak height

To establish whether or not a significant difference existed between the buprenorphine concentration of the sample injections, a Kruskall-Wallis test was performed ($p > 0.05$). Since the p-value was greater than 0.05, it was concluded that no significant difference existed between the buprenorphine concentration of any of the injections (including the unfiltered) and thus no further statistical analysis was required. However, by observation it was clear that some of the 1ml buprenorphine solution made up was lost (presumably retained by the filter) after filtration. Therefore, the above data cannot be used to draw any conclusions about whether or not the injections will have the same psychoactive effect on injection since the final volume of the injections and therefore the amount of buprenorphine they contained was not known.

Use of filters

Table 8: Subjective comments on the use of filter

Filter	Comments
Cotton	Very easy and convenient to use but very soft so needle can easily penetrate to other side resulting in no filtration. Injection appears cloudy. ~0.1ml retained by cotton.
Cigarette	Quick and easy to use. Leaves significant amount of white residue on spoon. Retains some drug solution. Injection cloudy.
Rizla	Easy, quick and convenient to use. Retains some solution but less than cigarette and cotton. Injection very cloudy. Filter thicker so more difficult to penetrate.
Dental	Difficult to penetrate but leaves white residue on spoon.
Steribox 1	Inefficient at drawing up solution from spoon, takes a few attempts. Leaves lots of white residue on spoon. Time consuming.
Millipore	Most time consuming to draw up as seems to introduce air bubbles. Sometimes break when attempts made to remove bubbles. Undissolved drug accumulates on filter but is easily redissolved. Quite clear injection. Damaged after use.
Pall	See Millipore.
Acrodisk	Difficult to draw up all solution as no needle. Time consuming as need to remove filter before eliminating air bubbles. Low final injection volume so assume retained by filter.

Discussion of results

Particle count

It has been shown that filtering buprenorphine injections results in a decrease in the total number of particles present. From the statistical analysis, it would appear that injections prepared with a cigarette filter do not contain a significantly lower number of particles than unfiltered injections. As shown in figure 4 the Acrodisk, prototype (Pall and Millipore) and Steribox 1 filters are far more effective at reducing the

number of particles in an injection of buprenorphine than the makeshift Rizla, cigarette and cotton filters, producing a reduction in particle count of 90 – 100%. Although this is an important finding which could be put to use in practice, it should be borne in mind that only particles within the size range 2 - 60µm were counted and some particles outside this range may have been present but not counted.

The Tukey's analysis results indicate that the dental filter is just as effective as the commercial and prototype filters at reducing the injection particle count. This was unexpected as the dental filter was a makeshift filter as it is not designed for filtering injections. However, the composition of the dental filter should perhaps be taken into account. As stated in table 1, the filter consists of layers of cotton wool rolled in thin layers with the outer layer fixed with a neutral bonding agent. The drastic reduction in particle number may therefore be due to the compact nature of the filter which allowed it to act as an efficient barrier to particles. On the other hand, it was noted that during preparation of the injections with the dental filters, a considerable amount of white residue was left on the spoon or Stericup. There is a possibility that this residue was undissolved drug which would have produced a misleadingly low particle count or could be from the filter, further work would be required to confirm this. Subutex ® tablets are designed to dissolve under the tongue and should therefore be fully soluble.

A decrease in the size of particles injected in addition to the number of particles injected is desirable after filtration. The majority of the filters tested caused a shift in the size distribution of the particles present in the filtered injections towards the smaller end of the scale. However, the cigarette, Rizla and cotton filters actually have a larger proportion of particles greater than 5µm in diameter than the unfiltered

injections. A possible explanation for this is that due to the fibrous nature of these filters, large fibres may have been shed into the injection from the filter causing an increase in the proportion of particles measuring more than 5µm in the sample. The Millipore filter was the most effective at shifting particle size distribution towards a smaller range, with approximately 90% of the particles in the Millipore injections measuring less than 5µm. The use of a filter that eliminates such a considerable proportion of large particles as the Millipore may substantially reduce the incidence of small vessel blockage and consequent medical complications. Further clinical work is required to confirm this. It is however wrong to assume that because an injection contains a high proportion of small particles that there is no risk of it causing blockage of vessels, risk cannot be removed.

Statistical analysis has shown that there is no significant difference between the number of particles in injections prepared using spoons and the number of particles in injections prepared using the complete injecting kit. However, although the environment in which the injections were prepared was not an aseptic one, good laboratory practice was observed throughout the investigation, that is, lab coats were worn and long hair was tied back, and so it was probably less contaminated than the environment used by IDUs. In addition, the spoons were carefully washed and dried between each injection to prevent cross-contamination of the samples, which may not be an accurate representation of most IDUs' behaviour. Conversely, the tea towel used to dry the spoons may have contributed to the particle count if fibres were shed. Therefore, in practice the use of a sterile injecting kit instead of spoons may have more of an impact on the number of particles contained in an injection than the data gathered implies and further work could be carried out to establish this. Despite

the lack of evidence that the use of an injecting kit reduces particle number, the possibility that it may reduce sharing of injecting paraphernalia and therefore the spread of blood borne viruses is reason enough alone to promote its use among IDUs.

Table 6 shows how the process of injection preparation can contribute to the particle count. It would be reasonable to predict that using fibrous makeshift filters such as cotton, Rizla and cigarette to prepare an injection would result in an injection containing a higher number of particles than injections prepared with the prototype membrane filters (Pall and Millipore). This is because such makeshift filters are more likely to shed fibres into the filtrate. However, as table 6 depicts, the opposite was the case. More work is required to establish whether or not the Pall and Millipore filters are in fact highly particle shedding or if the results occurred by chance or other factors. There are many other possible sources involved in the preparation of injections that could have introduced particles to the sample. For example, particles from the environment, the researcher or researchers clothing may have contaminated the injection.

Limitations of the method and further work

One limitation of the method was that, due to the sensitivity of the machine, only a 50µl sample of each injection was added to the beaker of isoton for analysis by the Multisizer. The particle count recorded therefore assumes that the sample analysed was representative of the whole injection. In addition to this, once the sample injections had been produced, they were injected into a plastic vial and left to stand until they were analysed, possibly causing some of the buprenorphine in the solution to settle. The samples were always removed from the vial and analysed as soon as

possible to try to decrease any inaccuracies that may have resulted from this, but the time that the injections were left in the plastic vials was not exactly the same for each sample. However, control injections were also subjected to this condition and so it should be accounted for when results re compared.

It was noticed that occasionally the background count for an injection was larger than the sample count, for example Millipore (spoon) 15.08 - 20.03µm. The calculated number of particles for this filter and size channel was therefore zero (see table 5). It should be pointed out that as with most analysis equipment, background 'noise' is sometimes detected by the Multisizer which cannot be separated from the parameter being measured (i.e. number of particles). This may have resulted in there being a smaller sample count than was actually true. A result of zero particles does therefore not necessarily mean that the filter was able to eliminate all particles within a particular size range but it does indicate that it was a very effective filter. As mentioned earlier, it is trends in the number of particles that are being looked at and the exact number of particles present is not of importance.

As mentioned above, a filter that filters out the majority of large particles does not necessarily produce an injection which poses no risks to health. Further work, such as the follow up of IDUs who have been given and shown how to use various filters, is required to find out the effects of different filters on the incidence of medical complications.

Concentration analysis

The results indicate that filtering buprenorphine injections does not affect their concentration. The results obtained must, however, be interpreted with caution. Although their concentrations were the same ($p > 0.05$), it was noticed by observation that the volumes of the filtered injections varied. This implies that the filters retained varying volumes of injection. Differing volume will obviously have an impact on the psychoactive effect of the injection. Therefore although the filter appears to have no effect on the buprenorphine concentration (mg/ml) of an injection, it may affect the psychoactive effects experienced by the IDU. Since the exact volumes of the filtered injections were not recorded, the comparative psychoactive effects of each cannot be determined from the above results. Further work examining the amount of buprenorphine retained in the filters is required.

Use of filters

The researcher found the makeshift filters the most convenient to use. It was relatively easy to draw up the injection solution through the filters and therefore the process was quick. However, it was noticed that these makeshift filters were also easily penetrated by the needle, which may lead to some of the injection not being subjected to filtration. Unfortunately, the filters that were found to be most effective at reducing particle number and size seem to be the most difficult and time consuming to use. Also noted was that the Pall and Millipore filters would be very difficult to reuse compared to the makeshift filters as they often become damaged during their first use. This is advantageous in that if sharing and reuse can be discouraged (i.e. the filter is ‘single use’) this should decrease the risk of BBV transmission. Conversely, some IDUs may be deterred from using these filters if they cannot be reused as they think they are wasting some of the drug, so further tests on filter drug retention is required.

Limitations of method and further work

A user based study is required to investigate the opinions of IDUs on each filter.

The prototype filters (Pall and Millipore) were found to be extremely effective at filtering out large particles but they were also found to be inconvenient to use compared to the makeshift filters. Further work could include trying to make these filters more ‘user-friendly’. Alternatively, IDUs could be given training on the appropriate use of the filters, which may become easier with practice.

Conclusion

From the results it can be concluded that filtering buprenorphine injections with any of the filters tested results in a decrease in the number of particles present. The more commercially produced syringe filters (Pall, Millipore and Acrodisk) produce a dramatic shift in the size range of the particles in the injections towards a smaller size range. Filtering buprenorphine injections was not found to have an effect on their concentration although it is inconclusive at this stage whether or not filtering significantly affects the psychoactive effects of the injection. The next step in this area of work should be to develop a robust method to determine this.

The makeshift filters which are most commonly used by IDUs (cigarette, cotton and Rizla) are the least effective at reducing the number of particles in a buprenorphine injection. IDUs should therefore be advised that although any form of filtering reduces particles, the syringe filters are more effective than makeshift filters. Of the filters that are being considered for inclusion in the Steribox 2 (Pall, Millipore and Steribox 1), the Millipore filtered injections contain the least number of particles as well as the highest proportion of particles measuring less than 5µm. The Millipore filter would therefore be the recommended choice for inclusion in the Steribox 2 and further study regarding clinical outcomes. The Acrodisk would be ideal for reducing injection particle content but it is an expensive option for supply as part of an injecting kit.

In conclusion, filtering buprenorphine injections is an important harm reduction measure for IDUs and could potentially prevent the occurrence of many medical

complications. However, the provision of injecting equipment alone is not enough. Education of IDUs on the risks of not filtering injections and sharing injecting paraphernalia is also important. In addition, it must be understood that just because an injection is filtered with an effective filter, the injecting process is not without risks. Prepared injections are not sterile. The ideal way to eliminate harm would be to discourage IDUs from injecting tablets.

Despite the success of the filters tested in reducing the number of particles in injections of Subutex, their effectiveness and efficacy may not be the same for more commonly used street drugs such as heroin. Heroin is less soluble than buprenorphine and this may have implications for both the number of particles in and the final concentration of filtered injections. Further work needs with other drugs, results cannot be assumed to be transferable.

References

1 Obadia Y *et al.* Injecting misuse of buprenorphine among French drug users. *Addiction* 2001; 96: 267-272.

2 Stein MD. Medical Complications of Intravenous Drug Use. *Journal of General Internal Medicine* 1990; 5: 249-255.

3 Tebbett IR. Analysis of buprenorphine by high-performance liquid chromatography. *Journal of Chromatography* 1985; 347: 411-413