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Short Communication

Newly designed instrument for desalination: Comparison with dialysis bag

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ABSTRACT

All desalination methods which are currently used have limitations. A newly designed instrument by Zare A.M. was tested and compared with currently used method in Razi Vaccine and Serum Research Institute(RVSRI). Samples of anti snake venom for desalination was provided by department of anti-serum, RVSRI. Parameters included in this study were time and water consumption during desalination process. PH, conductivity, Total protein, potency and dry weight of samples. Results reveals that complete desalination process by our newly designed instrument consumes only 4.30 hours and 80 litters of purified water as compared to currently used method of dialysis bag with average of 120 hours of time and 1920 litters of purified water($P<0.0001$). Tests for potency also showed similarity in quality of both the samples. In conclusion we strongly recommend using of our newly designed instrument in desalination of anti-snake serum.

***Keywords*:** Desalination, Ammonium sulphate removal, Anti-snake venom, Desalination instrument.

INTRODUCTION

One of the most important and critical step in biological material production is desalination. Using dialysis bag, ultra filtration, ion exchange and gel filtration process are common for this propose. All desalination methods which are currently used have limitations: for example, mixed-bed ion-exchange columns risk the loss of charged materials, precipitation of salt by a non-aqueous solvent can result in co-precipitation of oligosaccharides, and gel chromatography uses highly cross-linked packing in which some of our product may be trapped (Packer *et al* 1998) .

Since biological materials are sensitive and their biological activity can be reduced significantly, the method for desalination should not be time consuming. Desalination by dialysis bag is a time consuming process that can affect the activity of product. Ultra filtration also is not able to remove the salt from the product 100%. This method, also, is a costly process (Anfinsen & Anson 1966).

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To overcome this problem a new instrument was designed. This study was undertaken to see the efficacy of this instrument in desalination of antiserum as well as comparison of this method with traditional method of using dialysis bag for desalination.

MATERIALS AND METHODS

In Figure 1 showing the internal picture of desalination instrument used in this experiment. The mechanism of its function is dialysis of sample verses purified water. It contains two containers, one purified water and second for desalination sample. The sample interaction with purified water for ion exchange takes place by passing through dialyzing package with the help of two separate pumps. This system is closed system and no chance of bacterial contamination.

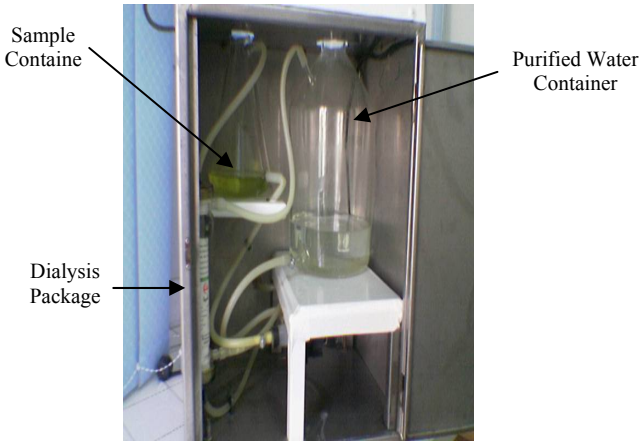

Figure 1: Internal Picture of Desalination Instrument

Sample Selection and analysis. For this study a sample of anti snake serum after precipitation with ammonium sulphate was divided into two equal portion. First portion was desalted by dialysis bag and the second portion was desalted by desalination instrument. Each portion of sample before desalination was tested for pH, conductivity, nitrogen content and total protein (Hanry 1986, Robert 2001), total protein (Hanry 1986), dry weight (Robert 2001) and after dialysis it was tested for the above mentioned parameters plus potency (Hazra 1945), and ammonium sulphate content (WHO 1986). During the desalination process every 20-30 minuets, dialysing water was tested for conductivity (Nordmann 1980) and it was changed with fresh purified water. Desalination process was considered to be finished when the conductivity of desalination samples reduced to less than 50µs and ammonium sulphate content was nil. Samples were frozen at -20 ^{o}C and stored until analysis. Amount of purified water consumption and time (hours) required to complete desalination process was recorded. This test was repeated six times to confirm the results and statistical analysis was carried out using Student t-test.

RESULTS AND DISCUSSION

The most important parameters in this study was comparison of time and amount of water consuming during desalination of product. As it is shown in table 1.the average time consuming for complete desalination was 120 hours by using dialysis bag while this time was reduced to 4.30 hours by using the instrument ($P<0.0001$) (Table 1). In Table 2, showing the purified water consumption. The average purified water consuming for complete desalination was 1920 litters by using dialysis bag which was reduced to 80 litters by using the our instrument ($P<0.0001$).

Sample dry weight. In Table 3, showing the amount of dry weight after desalination. Dry weight of sample by using dialysis bag was 0.17gm/cm^3 while by using instrument it was found to be 0.07gm/cm^3.

Table1. Time consumption: comparison during desalination process by both the methods.

Methods	Average time (hr)	Standard Error	P- value	Minimum time (hr)	Maximum time(hr)	Standard Deviation
Dialysis Bag	120	0.58	P<0.0001	96	144	16.97
Instrument	4.30	7.59	P<0.0001	3	6	1.3

Table2. Purified water consumption: comparison during desalination process by both the methods.

Methods	Average time (hr)	Standard Error	P- value	Minimum time (hr)	Maximum time(hr)	Standard Deviation
Dialysis Bag	1920	165.57	P<0.0001	1500	2500	370.14
Instrument	80	8.37	P<0.0001	60	110	18.71

Table3. Sample dry weight comparison after desalination process by both the methods.

Methods	Average dry weight (mg/cm^3)	Standard Error	P- value	Minimum dry weight (mg/cm^3)	Maximum dry weight (mg/cm^3)	Standard Deviation
Dialysis Bag	0.17	0.01	P<0.001	0.06	0.09	0.02
Instrument	0.07	0.01	P<0.001	0.14	0.18	0.02

Table4. Sample total protein after desalination process by both methods.

Methods	Average total protein (mg/ml)	Standard Error	P- value	Minimum total protein (mg/ml)	Maximum total protein (mg/ml)	Standard Deviation
Dialysis Bag	146.22	38.48	P<0.05	91.25	184	38.48
Instrument	78.44	23.45	P<0.05	50	106	23.45

Nitrogen content and total protein. Total protein of samples after desalination by both the methods were quite different. Samples desalted by dialysis bag contained almost twice amount of total protein as compared

Potency test. Since our samples for desalination were anti snake venom, the potency test of samples were applied on mice in order to confirm the functional activities with the method using our designed instrument.of samples. The results showed similar in potency by both the methods (Data were not shown). No activity was lost during desalination either by using dialysis bag or our designed instrument. This test was carried out in

poisonous animal department, Razi Vaccine and serum research institute, Iran.

All desalination methods which are currently used have limitations: for example, mixed-bed ion-exchange columns risk the loss of charged materials, precipitation of salt by a non-aqueous solvent can result in co-precipitation of oligosaccharides, and gel chromatography uses highly cross-linked packings in which some of our product may be trapped (Packer *et al* 1998).

In the method using our newly designed instrument the use of water for desalination and time required to finish the process of desalination reduced significantly. Reduction in water and time consumption in desalination process by our designed instrument is due to increased contact surface between deionized water and desalination sample. Reducing time and purified water consumption in the process of desalination is very important because it can reduce the risk of product microbial contamination, product denaturation, and finally production cost.

Using our newly designed instrument we found that the dry weight of biological product to be less than the dry weight of same sample using dialysis bag comparatively at the end of process. The difference in dry weight of sample after desalination can be due to pore size of the membranes or it be due to the difference in the pressure used in using instrument method. However since the potency of sample product was similar in both the methods it indicates that in the method of using instrument some of unwanted materials with very low molecular weight are removed during the desalination process. Hence this can be another advantage of using our newly designed instrument. Similarly we found that the total protein after the end of process in using our instrument is significantly less than the method using dialysis bag. As we know one of the most important factors in producing a good antivenin is high potency with low protein, hence as mentioned previously the reason can be due to loss of unwanted small molecules of peptides that were removed during desalination by instrument. Using biuret method of estimation for total protein also confirmed the results obtained by Kjeldal method. All other factors like PH and conductivity were similar in both the method, which are indicative of efficacy of our instrument.

In conclusion since the designed instrument has the advantages of being Portable and takes small place in any laboratory, Reduce the cost of desalination significantly by reduction in time and purified water consumption of process, system is packed and closed hence can be sterile, with out interfering the quality of product, it can be substituted with the old method of using dialysis bag for desalination of biological products. We also suggest more experiments on various biological products like enzymes to confirm the efficacy of our newly designed instrument.

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