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Comparative Study on Separation of Proteins from Whey Waste by Solvent Sublation in Batch and Continuous Mode

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Abstract

The work is based on investigation on the separation of proteins from whey waste collected from local confectionery. Separation phenomenon involves solvent sublation technique both in batch and continuous mode. The objective of this work was to make a comparative study of afore mentioned batch and continuous method. The effect of pH, initial feed, concentration of feed, nitrogen gas flow rate, surfactant concentration, ionic strength, concentration of organic solvent and temperature were investigated in details. The amount of protein recovered in single continuous mode after 5 hr was 3644 mg, %Rp (90%), total volume (8100 ml), effective concentration (49.5 mcg/ml). The VFR 14 cc/min, %Rp was nearly 1.3 times more in continuous solvent sublation technique in comparison to the batch mode at optimum pH and GFR.

Keywords: Volumetric flow rate; Solvent sublation; pH; Whey waste

Introduction

In cheese industry and sweet industry whey is produced as a by-product [1]. Whey contains number of essential proteins in about 5-10%, these proteins are either added to dairy products or animal fodder or are discharged, resulting in a high waste water burden. Whey becomes a strong pollutant when discharged into steams, its high organic matter enhances biochemical oxygen demand (BOD), ranging from 30 to 40 gm of oxygen per liter. There are various methods of utilizing or disposing of whey. Thus a costs effective method for the enrichment or isolation of such protein fractions is of high economic and ecological interest. Current applied techniques are ultrafiltration, gel filtration, ion exchange or precipitation and coagulation [2]. An alternative but still less investigative method for the enrichment of whey protein is called solvent sublation, belonging to Adsorptive bubble separation method [3]. The advantage of solvent sublation over adsorptive bubble separation method is that higher removal efficiencies are positive. The sublation process does mixer and phase separations, which is needed in the solvent extraction. Furthermore, the effluent water from a sublation column removes residual solvent. Handling is simple and expenses are cheap. So, the solvent sublation has the potential in the environmental pollution treatment. The whey proteins differs widely in their functional properties (solubility, gelation properties, dispersibility, water holding capacity, stability, adhesion, emulsification properties, film formation, foaming properties, organoleptic properties, viscosity, binding properties etc.) [4]. The functionality of protein is basically dependent on the molecular structure. There are various factors come variability in functional properties of whey proteins are the source of whey, season-dependent variation of its components, different conditions like pH, ionic strength, heat treatment, and the presence of minerals [5]. Among the three major proteins in whey like alpha-lactalbumin (α-la), beta-lactoglobulin (β-lg), and bovine serum albumin (BSA), α-la has good emulsifying properties but its gelation ability is poor. β-lg has excellent gelling and foaming properties. Whey waste in batch foaming process gives a small amount of whey waste recovery at a time [6,7]. Solvent sublation method is easier to operate than foam fractionation since latter involves the product as mixture of protein and surfactant. A further study is required to separate the protein from protein surfactant complex. In solvent sublation collector sodium dodecyl sulphate (SDS) was not used, so protein is in pure form had been accumulate at the interface and finally protein was settle in the solvent chamber as white soft material. It was easy to collet it. It did not require evaporation of solvent to collect product. This solvent acts as blanket so protein did not dry up at the top aqueous layer. Through solvent was used repeatedly but it is costly. Efficiency may be enhanced in solvent sublation method by dispersing coalescence intermittently throughout the liquid column, by using finer sparger, so that effluent concentration is sufficiently low.

A little investigation has been done by solvent sublation method. Few researchers investigated solvent sublation of pure proteins (BSA, β-lg, α-la, latoferrin, lectoperoxidase, lusozyme) in synthetic feed to see the effect of pH, and the volumetric flow rate with solvent concentration. Wu et al. [8] investigated on solvent sublation of L-lusine and found that it can be used to separate the low concentration L-lysine from the aqueous solution by using dodecyl benzene sulforc acid as surfactant and di-phospheric acid as extractant, n-heptane as the extractant solvent. He found the separation efficiency of solvent sublation was higher than that of traditional foam fractionation and solvent extraction method. Separation of penicillin G from fermentation broth, was investigated by Dong et al. The flotation product was quantitatively analysed by HPLC and compared with traditional solvent extraction. The effect of pH of the solution, NaCl concentration, concentration of butyle acetate (BA) in organic phase were investigated in details. Solvent extraction, flotation complexation extraction was proposed first time by Dong et al. [9] This new technique was used to separate and purify L-phenylalanine from fermentations liquid with good results. The flotation product from the fermentation liquid under optimal conditions, after back-extraction and re-crystallization was characterized by FTIR and HPLC, and its

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purity was more than 98%. Martinez-Araganet et al. [10] thoroughly studied about the different solvents which can be used in extraction of proteins by solvent sublation method, they found out that such solvents which breaks the disulfide bond in potent that should not be used a stability this of proteins as may the bond cause helps to lower retain tertiary structure and thereby prevent unfolding of the proteins, so chloroform and alcohols are not advised to use. They also proposed that any organic solvents which is less toxic having higher interfacial tension value and with a logP value greater than 4 can be suitable used as solvent for carrier mediated extraction proteins.

The aim of the present work was to make a comparative study on separation of protein as a whole by solvent sublation batch and continuous method. Both the batch and continuous method of solvent sublation was performed under the effect of several parameters like; pH, volumetric flow rate (VFR) etc. [11,12]. But its application is still continuing. Its feasibility at various operating conditions had been elucidated. The role of pH and volumetric flow rate on the performance characteristics were the prime objective here on the comparative study of both batch and continuous mode of solvent sublation in separation of protein from whey waste.

Materials and Methods

Whey waste was supplied by a local confectionary. Octanol (i-octanol) was obtained from Merck Ltd. India. Double distilled water was prepared at the laboratory. The instruments used were: UV- spectrophotometer (UV-1700 Shimadzu), pH meter (Satorious), Peristaltic pump with volumetric flow rate controller (RIVOTEK, India). Ultrasonic cleaner (Takashi), Digital weighing balance (Sartorious). Solvent sublation glass column with fritte (Remco Ltd, local glass fabricator).

Preparation of whey filtrate

Commercial whey was obtained from local sweet shop, it was then filtrated using a cheese cloth and filtrate was then poured in centrifuged tube and was subjected to centrifugation at 500 g for 30 min. The filtrate was collect through whatman filter paper-4 and again centrifuged in the same manner for 15 min and then the process was repeated till it gives constant OD value at λ_{max} of 280 nm. The filtrate was kept in a glass container inside the refrigerator to be used for subsequent experiments. The filtrate whey sample (40 ml) was taken in a Petridis. The Petridis was kept in BOD at the temperature of 50°C over night for drying. After drying a solid layer was found on Petridis, which was taken by spatula and weight was taken. From the difference of weight the amount of powder whey was found. It was found that every 40 ml of whey gives 1.25 gm of dried powder.

Solvent sublation (Batch process)

The apparatus (Figure 1) consists of a long column of 1 meter height that is fitted by frit at the bottom and an enlarged solvent chamber at the top. The solvent chamber is fitted with a reflux condenser. The column shows inlet, outlet for feed and effluent. The solvent chamber shows also inlet and outlet for solvent. The main glass apparatus is assembled with a nitrogen cylinder, rotameter (gas flow rate controller) Feed of desired concentration was prepared then the pH of the feed was adjusted as per requirement. The column was then filled with feed solution. The level of aqueous feed reaches 1 cm below the top of this column. The enlarged part of the solvent chamber was then covered with the glass cover. Required volume of Octanol was poured inside the chamber and a clear feed/octanol interface was visible. Nitrogen gas was passed through the feed at desired gas flow rate (GFR). On the

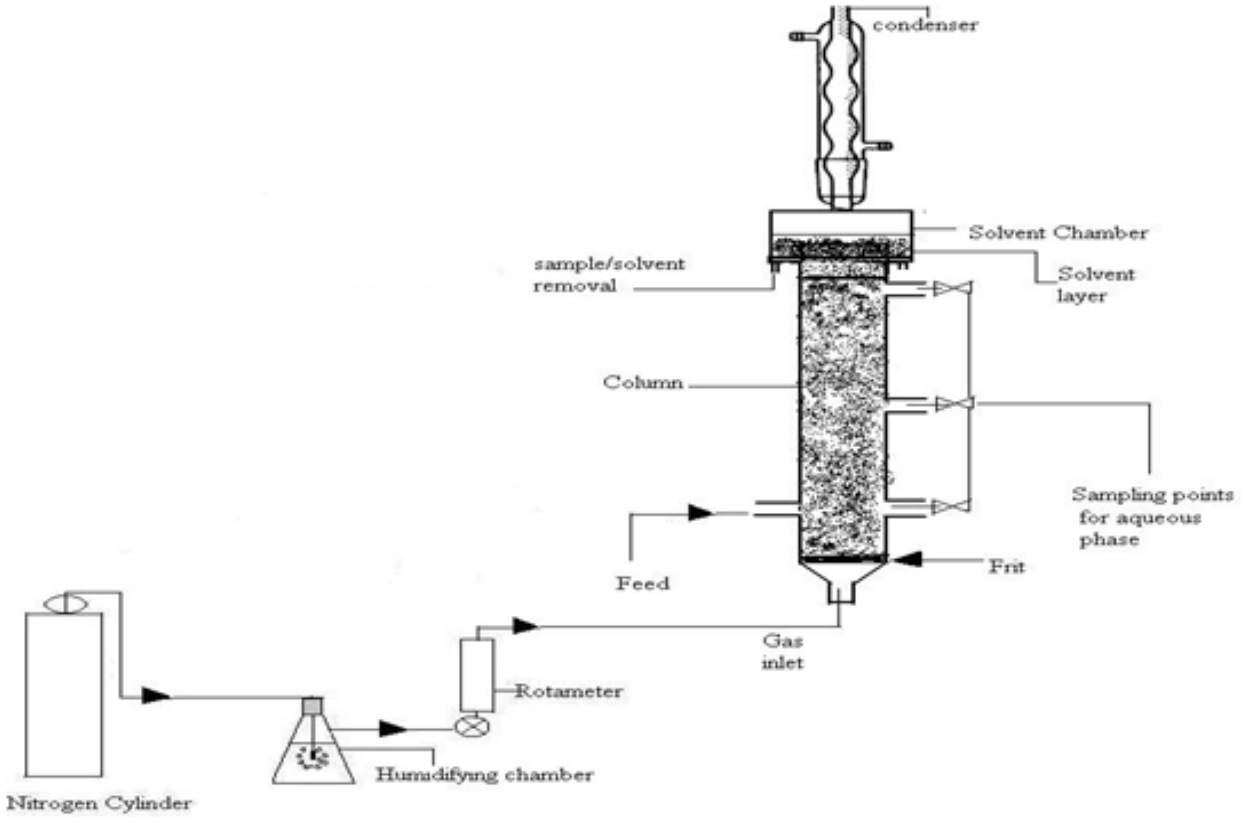

Figure 1: Schematic diagram of Solvent Sublation Apparatus operating in Batch Mode.

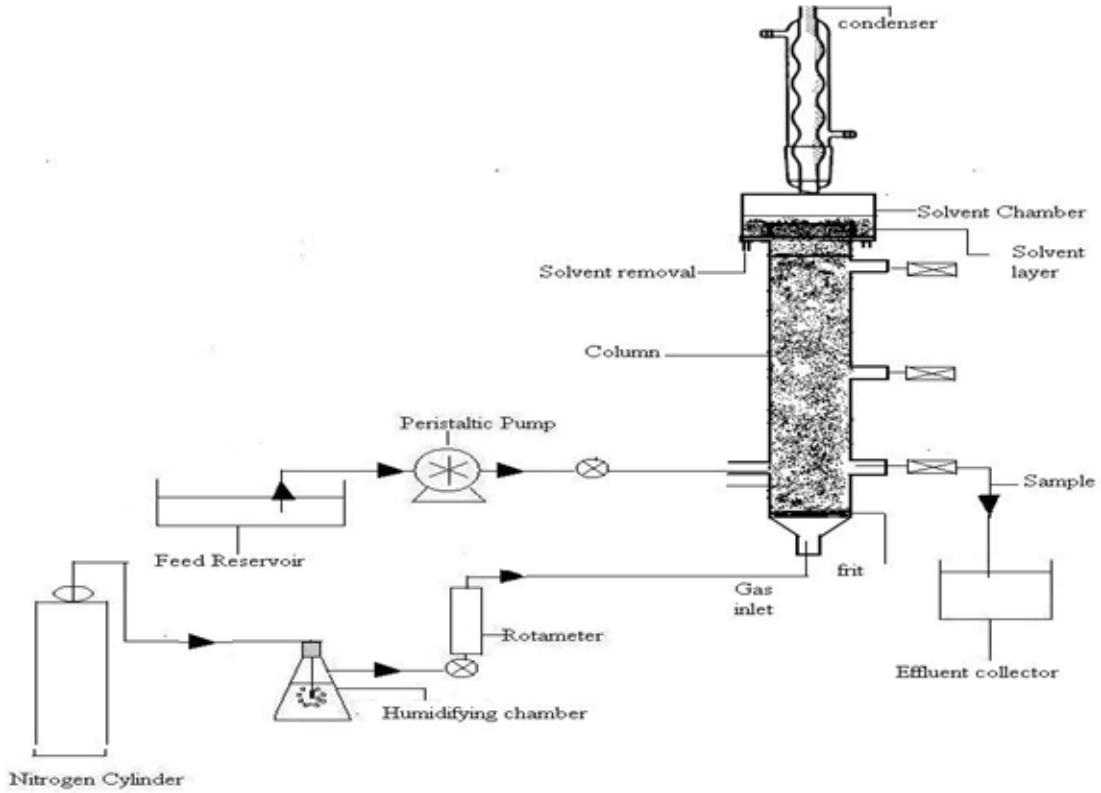

Figure 2: Schematic diagram of Solvent Sublation Apparatus operating in Continuous Mode.

Time (min)	Volume of Feed sample (ml)	Residual feed volume (ml)	Residual feed conc (mcg/ml)	Amount in residual feed (mg)	Amount separated (mg)	%RP	$t_{1/2}$ (min)
30	5	3895	449.5	1750.80	199.20	10.21	
60	5	3890	394.5	1534.60	415.40	21.30	
90	5	3885	342	1328.67	621.33	31.86	
120	5	3880	287	1113.56	836.44	42.89	146
150	5	3875	232	899	1051	53.89	
180	5	3870	177	684.99	1265.01	64.87	
210	5	3865	174.5	674.44	1275.56	65.41	

Initial volume of feed=3900 ml, Amount of protein in initial feed=1950 mg

Table 1: Performance criteria of whey in Batch mode under following condition (solvent sublation) Ci-500 mcg/ml, pH-2, GFR-175 cc/min.

top of the solvent chamber a condenser was connected to prevent the evaporation of solvent Residual liquid was collected from outlet at fixed intervals and was immediately analyzed. After sometime steady state concentration of effluent was observed. The experiment was continued upto 3 hours. At the end of run the total volume of residual liquid was collected and analyzed. The total input amount, output amount, loss amount, recovery %, were also calculated and tabulated [8].

Solvent sublation (Continuous process)

The glass column and its assembly description are same as Figure 2. a reservoir, peristaltic pump attached to supply feed to the column continuously at a desired volumetric flow rate (VFR). After loading the column with desired concentration of feed the top of the column was then covered with the receptacle (solvent chamber). Required volume of octanol was poured inside the chamber and a clear feed/octanol interface was visible. Feed was supplied from outside though a inlet in the column with the help of a peristaltic pump to maintain a constant volumetric flow rate, The flow rate of the outgoing effluent was same as the incoming feed. The effluent was continuously collected and analyzed. Nitrogen gas was passed through the feed at desired gas flow rate (GFR). After sometime the effluent showed steady concentration. The experiment was continued till 5 hours. The total effluent volume. residual volume was measured at the end of the run and was analyzed. The total input amount, output amount, loss amount, recovery %, were also calculated and tabulated.

Results

The present work deals with the comparative study of the separation and removal of whey proteins from the commercial whey waste (which is more often discarded as a waste product in sweet industry) by both batch and continuous solvent sublation method. Both batch and continuous solvent sublation mode of operations were performed and effect of several parameters such as pH, volumetric flow rate (VFR), Gas flow rate (GFR) on the separation process were studied thoroughly.

Effect of pH

From the values obtained in Tables 1-3 in batch mode of solvent sublation and comparing them with Figure 3, in batch mode, it was

Time (min)	Volume of Feed sample (ml)	Residual feed volume (ml)	Residual feed conc (mcg/ml)	Amount in residual feed (mg)	Amount separated (mg)	%RP	$t_{1/2}$ (min)
30	5	3895	447	1741.06	208.94	10.71	
60	5	3890	392	1524.88	425.12	21.80	
90	5	3885	332	1289.82	660.18	33.85	
120	5	3880	272	1055.36	894.64	45.87	137
150	5	3875	214.5	831.19	1118.81	57.37	
180	5	3870	157	607.59	1342.41	68.84	
210	5	3865	154.5	597.14	1352.86	69.37	

Initial volume of feed=3900 ml, Amount of protein in Initial feed=1950 mg

Table 2: Performance criteria of whey in Batch mode under following condition (solvent sublation) Ci-500 mcg/ml, pH-5, GFR-175 cc/min.

Time (min)	Volume of Feed sample (ml)	Residual feed volume (ml)	Residual feed conc (mcg/ml)	Amount in residual feed (mg)	Amount separated (mg)	%RP	$t_{1/2}$ (min)
30	5	3895	449.5	1750.80	199.20	10.21	
60	5	3890	402	1563.78	386.22	19.80	
90	5	3885	352	1367.52	582.48	29.87	
120	5	3880	299.5	1162.06	787.94	40.40	157.7
150	5	3875	252	976.5	973.5	49.92	
180	5	3870	202	781.74	1168.26	59.91	
210	5	3865	199.5	771.07	1178.93	60.45	

Initial volume of feed=3900 ml, Amount of protein in initial feed=1950 mg

Table 3: Performance criteria of whey in Batch mode under following condition (solvent sublation) Ci-500 mcg/ml, pH-8, GFR-175 cc/min.

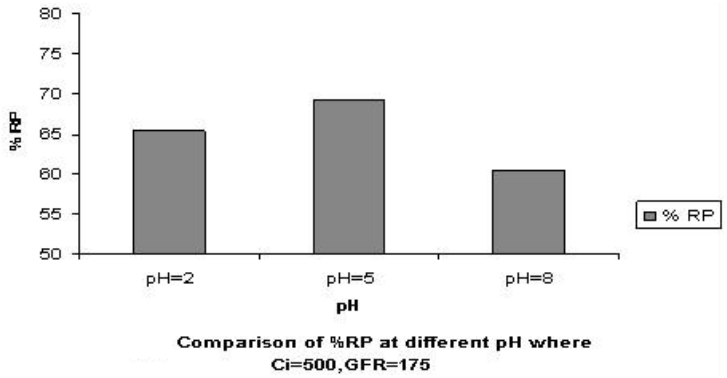

Figure 3: Solvent Sublation- Batch Process.

Time (Min)	Effluent concentration(mcg/ml)	Eff+Res Vol (ml)	Input amount (mg)	Output amount (mg)	Loss amount (mg)	Separated amount of whey (mg)	%RP
30	104.5						79.10
60	99.5						80.10
90	92						81.60
120	94.5						81.10
150	89.5	7433	3750	3724.83	25.16	3074	82.10
180	89.5						82.10
210	89.5						82.10
240	87						82.60
270	89.5						82.10
300	87						82.60

Table 4: Performance criteria of whey in continuous mode under following condition (solvent sublation) Ci-500 mcg/ml, pH-5, GFR-175 cc/min, VFR=12 ml/min.

found that the recovery process was optimum at pH 5, at lower pH 2 at the performance criteria was lesser and still least at a higher pH 8 than the optimum. The enrichment ratio (E_R) (maximum at pH 5) increased with the increased in pH and then decreased above pH 5. This is due to the fact that the isoeletric pH of the whey protein is around 5, bellow which it becomes negative charge. Since it is no more ionic (net charge is zero), so it favours interface than the bulk liquid (aqueous). It seems that whey proteins may be separated from the bulk to adsorption of protein in the interface at IEP [13].

Effect of GFR

In continuous mode of solvent sublation range of GFR was 150-200

Time (Min)	Effluent concentration (mcg/ml)	Eff+Res Vol (ml)	Input amount (mg)	Output amount (mg)	Loss amount (mg)	Separated amount of whey (mg)	%RP
30	59.5						88.10
60	54.5						89.10
90	52						89.60
120	52						89.60
150	49.5	8030	4050	4044.0475	5.9525	3644	90.10
180	49.5						90.10
210	49.5						90.10
240	49.5						90.10
270	47						90.60
300	49.5						90.10

Table 5: Performance criteria of whey in continuous mode under following condition (solvent sublation) Ci-500 mcg/ml, pH-5, GFR-175 cc/min, VFR=14 ml/min.

Time (Min)	Effluent concentration (mcg/ml)	Eff +Res Vol (ml)	Input amount (mg)	Output amount (mg)	Loss amount (mg)	Separated amount of whey (mg)	%RP
30	149.5						70.10
60	139.5						72.10
90	137						72.60
120	139.5						72.10
150	134.5	8627	4350	4297.995	52.00	3174	73.10
180	134.5						73.10
210	134.5						73.10
240	129.5						74.10
270	132						73.60
300	129.5						74.10

Table 6: Performance criteria of whey in continuous mode under following condition (solvent sublation) Ci-500 mcg/ml, pH-5, GFR-175 cc/min, VFR=16 ml/min.

Time (Min)	Effluent concentration(mcg/ml)	Eff+Res Vol (ml)	Input amount (mg)	Output amount (mg)	Loss amount (mg)	Separated amount of whey (mg)	%RP
30	59.5						79.10
60	54.5						82.10
90	52						84.10
120	52						84.10
150	49.5	8026	4050	3758.8495	291.1505	3359	83.10
180	49.5						83.10
210	49.5						83.10
240	49.5						84.10
270	47						83.60
300	49.5						83.10

Table 7: Performance criteria of whey in continuous mode under following condition (solvent sublation) Ci-500 mcg/ml, pH-5, GFR-150 cc/min, VFR=14 ml/min.

Time (Min)	Effluent concentration(mcg/ml)	Eff +Res Vol (ml)	Input amount (mg)	Output amount (mg)	Loss amount (mg)	Separated amount of whey (mg)	%RP
30	74.5						85.10
60	62						87.60
90	64.5						87.10
120	64.5						87.10
150	67	8026	4050	4045.092	4.908	3504	86.60
180	67						86.60
210	67						86.60
240	62						87.60
270	67						86.60
300	67						86.60

Table 8: Performance criteria of whey in continuous mode under following condition (solvent sublation) Ci-500 mcg/ml, pH-5, GFR-200 cc/min, VFR=14 ml/min.

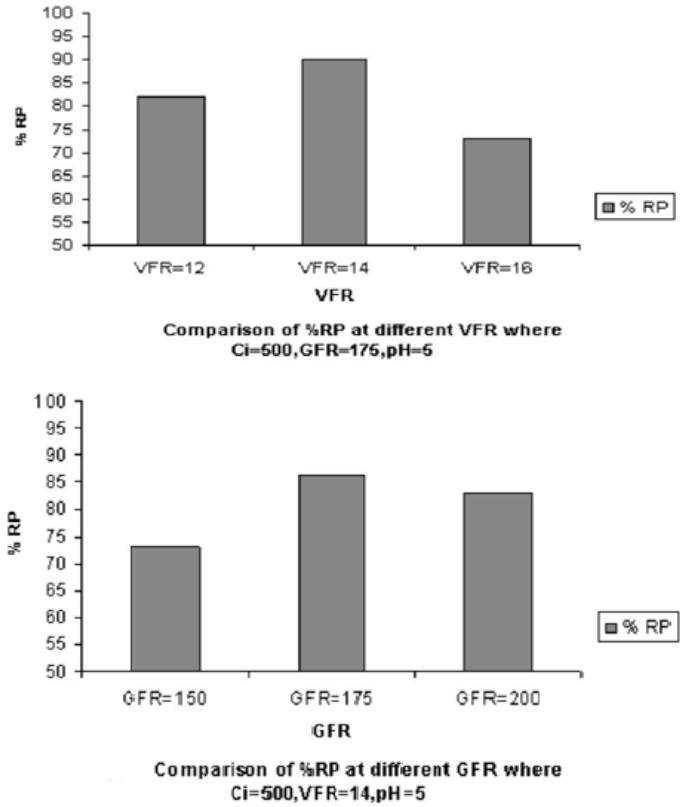

Figure 4: Solvent Sublation- Continuous Process.

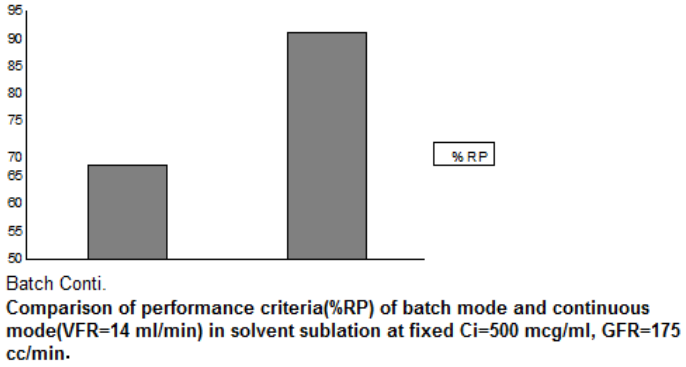

Figure 5: Solvent Sublation- Batch vs Continuous.

cc/min, %Rp increased with the increased of GFR up to 175 cc/min and then it decreased at 200 cc/min. At high GFR bubbles coalescence and decrease in interfacial area caused low %Rp. Therefore, GFR at 175 c/min was found suitable. From the values obtained in the Tables 4-8 in continuous mode of solvent sublation and compare them with Figure 4, its was found that the performance criteria was highest at a GFR 175 cc/min.

Effect of VFR

From the vales obtained in Tables 4-6 in continuous mode and comparing them with Figure 5, it was found that the optimum volumetric flow rate was 14 ml/min. It was observed that at a higher VFR the performance criteria showed lower value when compare to that of a lower VFR. %Rp increased with the increased of VFR up to 14 ml/min. With increased of input liquid %Rp increased up to a limit. At higher VFR (16 ml/min) residence time of input liquid is less and some of protein left the column without getting adsorbed. Therefore, effluent showed high concentration.

Effect of recovery process

From the Figure 4, it was found that in continuous mode at VFR 14 cc/min, %Rp was nearly 1.3 times more than that of batch mode at optimum pH and GFR. From the values obtained in the Tables 1-3 in batch process with various pH and Tables 4-6 at various VFR in continuous mode, it was observed that at any given condition the percentage recovery was greater in continuous operation mode at pH 5. The optimum operation conditions were found as GFR 175 cc/min, pH 5, VFR 14 ml/min. It is obvious that rate of separation of protein

rom whey is found higher in continuous mode than the batch mode. In solvent sublation method, amount of protein recovered in single stage of continuous mode after 5 hours operation was 3644 mg, %Rp 90%, total volume treated was 8100 ml and effluent concentration was 49.5 mcg/ml, at the optimum condition (Table 5).

Conclusion

It was observed that solvent sublation is useful method either to concentrate protein from dilute solution of whey or to decrease the protein lable of waste solution buy both batch and continuous mode operation. The method was found more effective in continuous mode of VFR 14 ml/min, GFR 175 cc/min at pH 5. The enrichment ratio almost 1.5 times in continuous operation than that of batch mode operation. %Rp was also nearly 1.3 times more than that of batch mode at optimum pH and GFR. Optimisation of data adapting a suitable model may provide more precise operating conditions for the maximum recovery of proteins.

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