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Recovery of Gentamycin Sulphate from Dilute Solution by Adsorptive Bubble Separation Method

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The aim of this work is to study performance criteria of separation of gentamycin from aqueous solution by controlling of different variables and surfactants in adsorptive bubble separation method. Separation and purification of drug component from a mixture of components, separation of enantiomeric drugs mixture, separation of chemical constituents from plant source and removal of drug components from wastewater is carried out through foam separation method. The result showed that gentamycin sulphate can be easily separated from dilute solution of drug mixture in a short time. The influence of active variables including concentration of surface active agent, pH, superficial gas velocity (SGV) and recovery of drug were investigated. The optimum separation has been set as follow: concentration of surface active agent dioctyl sodium sulfosuccinate (DOSS) (1.0 mM) and sodium lauryl sulphate (SLS) (1.0 mM), pH 5, superficial gas velocity 0.054 cm/s and drug recovery 69.55 and 80.32, respectively. The unique advantage of the present work relative to other reported method is the higher separation efficiency and lowest cost.

Key Words: Foam separation, Gentamycin sulphate, Surface active agent, Aminoglycoside, Dioctyl sodium sulfosuccinate, Sodium lauryl sulphate.

INTRODUCTION

Foam fractionation is applicable to performed in order to study the prospects of enrichment of active principles from some drugs and surfactants that are of surface activity. Recently, an experimental approach using foam separation method to separate penicillin G from drug mixture and surface active agent is developed[1]. This investigation led to the conclusion that separation, enrichment and recovery of drug (penicillin G) from dilute solution can be done successfully at low cost. The applicability of foam separation method to separate out of gentamycin sulphate from its dilute solution using dioctyl sodium sulfosuccinate (DOSS) and sodium lauryl sulphate (SLS) as collector surfactant has been investigated. Adsorptive bubble separation methods (ABSM) is among the less familiar separation methods. The principle of

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ABSM is based on differences in properties of the materials to be separated. Foam separation is a separation method belonging to the ABSM[2]. In foam fractionation, dissolved material is selectively adsorbed on the surface of rising bubbles and then is partially segregated by the foam. This fundamental approach has been stimulated by a great recognition of the potentialities of this technique possess to become an attractive replacement to more costly separation techniques[3-5]. Foam separation shows particular promise for being environmental friendly, energy saving and economical in terms of fixed and running costs means, for removing substances present at low concentrations from large volumes of liquids[6].

Foam separation is essentially governed by mass transfer phenomena that occurs under flowing conditions and is characterized by diffusion in the bulk of the liquid and adsorption at the gas-liquid interface of the bubbles. Therefore, a successful separation must take into account several parameters and conditions. The separation of materials from one another by foam separation lays on their physico-chemical properties, equipments and mode of operation used, as well as conditions in which the process occurs[7]. While the physico-chemical properties of the materials determine those materials capacity to be separated by foam fractionation, the right choice of types of columns and auxiliary devices used, together with operational conditions, are of paramount importance to achieve an optimized enrichment. Drugs containing the surface activity under investigation and on the process of foam fractionation. The equilibrium adsorption of a dissolved material at the gas-liquid interface is given by Gibbs as the equations:

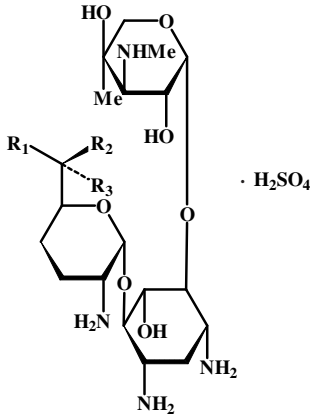
$$d\gamma = - RT \Sigma G \text{ id } \ln a_i \quad (1)$$

where 'γ' = surface tension; 'R' is the gas constant; 'T'= absolute temperature; 'i' = surface concentration of the component 'i'; 'a_i' = activity of the component i.

$$\Gamma = -\frac{1}{R \cdot T} \cdot \frac{d\gamma}{d \ln C_B} \quad (2)$$

where Γ = surface concentration of surfactant; C_B = concentration of surfactant in the bulk.

Gentamycin sulphate is a mixture of sulphates of antimicrobial substances produced by *Micromonospora purpurea*. Gentamycin is highly ionized and pH varies between 3.5 to 5.5 in 40 % w/v solutions in carbon dioxide free water. It is neither absorbed nor destroyed in the g.i. t. However, absorption from injection site in muscles is rapid. It is distributed only extra cellular: volume of distribution (0.3 L/kg) is nearly equal to the extracellular fluid volume. It attains low concentration in serous fluids like synovial, pleural, *etc*. Plasma protein binding is clinically insignificant. Gentamycin is not metabolized and excreted unchanged in urine. Glomerular filtration is the main channel but tubular secretion and reabsorption are negligible. The plasma $t^{1/2}$ is 2-4 h. Gentamycin sulphate is easily separated by foam separation method with the help of dioctyl sodium sulfosuccinate (DOSS) and sodium lauryl sulphate (SLS) used as collector.



Structure of gentamycin sulphate

EXPERIMENTAL

Gentamycin sulphate was a gift sample from Greenco Biological Pvt. Ltd. Salt Lake, Kolkata. Dioctyl sodium sulfosuccinate (DOSS) and sodium lauryl sulphate (SLS) and Nile blue indicator was purchased from E. Merck (India) Limited. Antibiotic assay medium was purchased from Merck (India) Limited. Foam separation glass column was fabricated by local glass blower. Stalagmometer was purchased from local manufacturer and Shimazdu-1700 U.v/Visible Spectrophotometer was purchased from Hitachi Ltd., Tokyo, Japan. Digital pH meter (model MK- VI) - Systronics was purchased from E. Merck (India) Limited. Mercuric nitrate and nitric acid were used to estimate gentamycin sulphate, those were purchased from E. Merck (India) Limited.

Glass column with an internal diameter of 4.2 cm was used in this study. A 100 mL feed solution of desired concentration was prepared by dissolving drug and surfactant. Ratio of drug mole was varied in case of mixture of drugs that is added with surfactant solution. The pH was adjusted by using either 0.1 M NaOH or 0.1 M HCl. The drug solution was contacted with the gas bubble rising from the frit fitted at the bottom of the column. Nitrogen gas was passed through the bottom of the column *via* a gas flow meter and a humidifier. The surfactants, dioctyl sodium sulfosuccinate (DOSS) and sodium lauryl sulphate (SLS) form stable foam and drug was adsorbed on the foam-bubble interface. As the foam ascends the column, the liquid present in the inter bubbler space decreases due to drainage of liquid downward, while drug concentrate in the interface of gas bubble and liquid. The foam was allowed to overflow the top of the column into a container and collapse and then its drug concentration was measured by UV absorbance using a spectrophotometer. The concentration of initial feed solution and the residual solution were also determined by the same method. All the experiments were performed at room temperature which varied throughout the period of this study. No such variation of experimental data with temperature had been found.

Microbial assay of gentamycin sulphate (standard solution): Potency of the drug was assayed by conventional cup-plate method by using *Micrococcus lutea* (ATTC 9341) as test organism. *M. lutea* was maintained on antibiotic assay media I. For preparation of suspension of test organism, the agar was inoculated with *M. lutea*. The slant was then incubated at 37 ºC. The growth of fresh subculture (18 h) was washed with 3 mL sterile distilled water to make a suspension. Out of this 1 mL suspension was drawn aseptically and added to 100 mL molten medium (Antibiotic assay medium II).

Preparation of assay plate: 100 mL of assay medium in 250 mL flask was melted in water bath and allowed to cool down to *ca.* 45 ºC, 1 mL suspension of *M. lutea* was added to 100 molten assay medium, which was then mixed well and poured into 5 sterile Petri dishes (100 × 20 mm) aseptically. Agar medium was allowed to distribute uniformly and solidified. After solidification of medium in petri dishes, circular holes were cut on the medium with the help of the sterile cork bores having a dia of 8 mm. To wells 1 drop of the drug standard solution of five concentrations were added these plate were incubated at 37 ºC for 18-24 h.

Preparation of stock solution of standard: Stock solution of the drugs were prepared by dissolving 25 mg of the drug in 25 mL with phosphate buffer (pH = 8.0). Five concentrations 64, 80, 100, 125, 156 µg/mL was made.

Determination of unknown sample: The concentration was determined with the help of standard curve drawn. In an assay plate out of five plate holes, three were filled with known concentration of reference standard drug and the other two were filled with unknown sample. Triplicate plate for each concentration of standard solution were used along with one reference standard concentration in each late. Zone diameter of the unknown was plotted and interpolated on correlated standard curve. The unknown concentration of drug was determined from standard curve.

Experimental procedure for separation of surfactant and determination of critical micellar concentration: First step is to measurement of the critical missiler concentration (CMC) of those surfactants with the help surface tension methods. Then take the known concentration of those surfactant, sodium lauryl sulphate (SLS) and dioctyl sodium sulphosuccinate (DOSS) form stable foam through the help of saturated N_2 gas. Surfactants were adsorbed on the foam-bubble interface. As the foam ascends the column, the liquid present in the inter bubbler space decreases due to drainage of liquid downward, while surfactant concentrate in the interface of gas bubble and liquid. Then (initial, foamate and residual solution) concentration determined by titremetric assay method or UV absorbance using spectrophotometic methods.

Titremetric assay of dioctyl sodium sulfosuccinate (DOSS): It was pipette 10 mL of the sample solution into a 250 mL flask and 40 mL of chloroform was added, 50 mL of salt solution and 10 drops of bromophenol blue TS. Titrate against tetra-*n*-butyl ammonium iodide solution to the first appearance of a blue colour in the chloroform layer after vigorous shaking.

Titremetric assay of lauryl sulphate (SLS): 100 mL solution was prepared by dissolving 67.5 mg of SLS. It was transferred 20 mL to a separate flask and adds 15 mL chloroform and then it is added 10 mL dilute sulfuric acid and 1 mL diethyl yellow oracet blue-B solution. It was titrated with 0.004 M benzethonium chloride until the chloroform layer becomes green. Each mL of 0.004 M benzethonium chloride is equivalent to. 001154 g of sodium alkyl sulphate ($C_{12}H_{25}NaO_4S$).

Preparation of sample solution: It was transferred about 30.8 g of sample, previously dried at 105 ºC for 2 h and accurately weighed into a 500 mL volumetric flask, dissolves in chloroform. After diluted to volume with the same solvent and mixing.

RESULTS AND DISCUSSION

The present work deals with the separation and removal of gentamycin sulphate (GEN) with the help of surface active agents such as dioctyl sodium sulfosuccinate (DOSS, MW.444.6, CMC-2.75 Mm) and sodium lauryl sulphate (SLS, MW.288.38, CMC-8.0 Mm) by foam separation method. Surface active agents form micelles at the higher concentration and then surface activity decreases. So, amount of adsorption (%) of surface active agents in the interface decreases that causes lower values of enrichment (Er) and % of recovery (R) of any colligents. Since these drugs are surface active but not satisfactory of its surface activity, so these were removed/ recovered from dilute solution with the help of collector substances *i.e.* surface active agents.

Enrichment ratio (Er): It is the ratio of drug concentration in the foamate (Cf) and the drug concentration in the initial feed solution (Ci).

Separation ratio (Sr): It is the ratio of drug concentration in the foamate (Cf) and the drug concentration in the residual solution (Cr).

Percentage of recovery (%R): It is the percentage of the ratio of amount of the mass of drug in the foamate and the mass of drug in the initial feed solution.

Effect of concentration of surface-active agents on the enrichment and R values of drugs: It was observed from Tables 1 and 2, Fig. 1 ((Drug/SA; GEN/ DOSS), Fig. 2 (Drug/SA; GEN/SLS) that both Er, R values increases with the increase

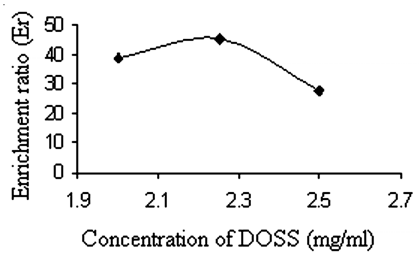


Fig. 1. Effect of concentration of SA (DOSS) on Er values of DG (GEN) at 36 ºC

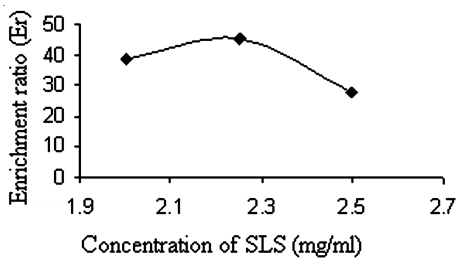


Fig. 2. Effect of concentration of SA (SLS) on Er values of DG (GEN) at 36 ºC

TABLE-1
PERFORMANCE CRITERIA OF SEPARATION OF DRUG (GENTAMYCIN) WITH COLLECTOR SURFACE ACTIVE AGENT (DOSS) AT DIFFERENT TEMPERATURE, AT DIFFERENT pH AND SUPERFICIAL GAS VELOCITY (SGV)

Temp. (ºC)	DG/SA in FD (mM/ mM)	FD, FM, RS Amount (mg)	pH	SGV (cm s^{-1})	Ci (mM)	Cr (mM)	Cf (mM)	Er	Sr	R (%)
33.0	0.2/0.8	20, 10.75, 5.80	5, 4.91, 4.41	0.040	0.2	0.05	4.48	22.40	89.60	53.80
33.0	0.2/0.8	20, 8.00, 5.38	5, 4.98, 4.45	0.054	0.2	0.06	2.75	13.75	45.82	40.00
33.3	0.2/1.0	20, 14.12, 5.94	5, 4.98, 4.51	0.040	0.2	0.05	5.33	26.23	71.60	43.05
33.3	0.2/1.0	20, 13.91, 5.81	5, 4.91, 4.51	0.054	0.2	0.05	6.95	34.75	139.00	69.55
33.3	0.2/1.2	20, 10.24, 6.00	5, 4.98, 4.41	0.040	0.2	0.06	3.41	17.05	56.83	51.20
33.3	0.2/1.2	20, 6.65, 6.01	5, 4.91, 4.54	0.054	0.2	0.06	2.66	13.30	44.33	33.25
33.3	0.4/0.8	40, 21.12, 9.91	5, 4.96, 4.54	0.040	0.4	0.10	9.60	24.00	96.00	52.80
33.3	0.4/0.8	40, 15.92, 7.23	5, 4.91, 4.51	0.054	0.4	0.07	6.92	17.30	8.85	39.80
33.5	0.4/1.0	40, 22.32, 10.21	5, 4.96, 4.50	0.040	0.4	0.10	12.40	31.00	124.00	55.80
33.5	0.4/1.0	40, 16.71, 8.13	5, 4.97, 4.50	0.054	0.4	0.08	11.93	29.82	149.10	41.77
33.6	0.4/1.2	40, 20.34, 10.00	5, 4.98, 4.45	0.040	0.4	0.10	7.26	18.15	72.60	50.85
33.6	0.4/1.2	40, 15.02, 9.12	5, 4.91, 4.61	0.054	0.4	0.09	5.36	13.40	59.55	37.55
33.6	0.6/0.8	60, 28.08, 17.32	5, 4.98, 4.70	0.040	0.6	0.17	7.58	12.63	44.58	46.80
33.6	0.6/0.8	60, 21.78, 10.81	5, 4.91, 4.30	0.054	0.6	0.11	4.84	8.06	44.00	6.30
33.7	0.6/1.0	60, 30.40, 17.89	5, 4.96, 4.60	0.040	0.6	0.18	25.33	42.21	140.70	50.66
33.7	0.6/1.0	60, 23.50, 11.00	5, 4.97, 4.60	0.054	0.6	0.11	19.58	2.63	178.00	39.16
33.3	0.6/1.2	60, 26.23, 15.13	5, 4.98, 4.50	0.040	0.6	0.15	8.19	13.65	54.60	43.71
33.3	0.6/1.2	60, 20.19, 10.21	5, 4.91, 4.40	0.054	0.6	0.10	6.73	11.21	67.30	33.65

TABLE-2
PERFORMANCE CRITERIA OF SEPARATION OF DRUG (GENTAMYCIN) WITH COLLECTOR SURFACE ACTIVE AGENT (SLS) AT DIFFERENT TEMPERATURE, AT DIFFERENT pH AND SUPERFICIAL GAS VELOCITY (SGV)

Temp. (ºC)	DG/SA in FD (mM/ mM)	FD, FM, RS Amount (mg)	pH	SGV (cm s^{-1})	Ci (mM)	Cr (mM)	Cf (mM)	Er	Sr	R (%)
34.3	0.2/0.8	20, 11.77, 5.81	5, 4.91, 4.86	0.040	0.2	0.050	4.90	24.50	98.00	58.85
34.3	0.2/0.8	20, 8.42, 5.49	5, 4.96, 4.51	0.054	0.2	0.050	2.93	14.65	58.60	42.12
34.5	0.2/1.0	20, 16.96, 5.91	5, 4.94, 4.59	0.040	0.2	0.050	4.73	23.65	78.83	52.12
34.5	0.2/1.0	20, 16.06, 4.02	5, 4.69, 4.75	0.054	0.2	0.050	8.03	40.15	260.70	80.32
34.6	0.2/1.2	20, 11.04, 5.91	5, 4.86, 4.15	0.040	0.2	0.060	3.68	18.40	1.33	55.21
34.6	0.2/1.2	20.0, 7.62, 5.00	5, 4.81, 4.10	0.054	0.2	0.050	3.04	15.20	60.80	38.72
34.4	0.4/0.8	40, 22.11, 9.96	5, 4.96, 4.40	0.040	0.4	0.100	10.05	25.12	100.50	55.32
34.4	0.4/0.8	40, 16.92, 7.00	5, 4.91, 4.11	0.054	0.4	0.070	7.35	18.37	5.00	42.32
34.6	0.4/1.0	40, 22.32, 10.21	5, 4.96, 4.65	0.040	0.4	0.100	12.95	32.37	125.70	58.31
34.6	0.4/1.0	40, 17.72, 8.03	5, 4.97, 4.50	0.054	0.4	0.080	12.65	31.62	158.10	44.31
34.4	0.4/1.2	40, 20.35, 8.12	5, 4.93, 4.40	0.040	0.4	0.080	7.62	19.05	95.25	53.39
34.4	0.4/1.2	40, 16.00, 7.00	5, 4.94, 4.31	0.054	0.4	0.070	5.71	14.27	81.57	40.00
34.8	0.6/0.8	60, 29.07, 17.31	5, 4.98, 4.51	0.040	0.6	0.179	7.85	13.08	43.85	48.85
34.8	0.6/0.8	60, 22.77, 10.89	5, 4.97, 4.40	0.054	0.6	0.110	5.06	8.43	46.00	7.95
34.7	0.6/1.0	60, 31.27, 18.00	5, 4.93, 4.50	0.040	0.6	0.180	26.05	43.41	144.70	52.12
34.7	0.6/1.0	60, 24.00, 11.01	5, 4.94, 4.50	0.054	0.6	0.110	20.00	3.33	181.80	40.00
34.4	0.6/1.2	60, 27.23, 15.13	5, 4.96, 4.39	0.040	0.6	0.150	8.50	14.16	54.48	45.57
34.4	0.6/1.2	60, 21.19, 10.20	5, 4.91, 4.30	0.054	0.6	0.100	7.06	11.76	67.23	35.32

of concentration of surface active agents in feed but decrease when concentration of surface active agents is increased to 2.5 mM at a particular experimental condition (fixed values of pH, SG V and Ci). At this optimum concentration of DOSS (1.0 Mm), the values Er, Sr, R of drug (PG) were respectively 34.75, 139.00 and 69.55 at pH 5, Ci of drug-0.2 mM at lowest SGV value. Similarly at optimum concentration of SLS (1.0 Mm), values of Er, Sr, R of gentamycin were obtained as 40.15, 260.7, 80.32, respectively at pH 5. These are slightly higher than DOSS.

Effect of pH on % recovery of drugs: It was observed from Tables 1 and 2 and Figs. 1 and 2 that maximum values of Er and R-values were observed at pH 5.The maximum enrichment and %R at lowest feed concentration (Ci of drug), lowest SGV values, at optimum pH value (5).

Effect of superfacial gas velocity (SGV) on the % removal and enrichment of drug: Gas flow rate was kept constant at a particular experimental condition by a air flow meter. They were fixed at 0.054 cm/s. High value of SGV causes low residence time of bubble in the column, so lesser amount of colligend/collector can be adsorbed in the interface and drug foam can not be collected. As a result, this is less enrichment of drug or colligend in the foamate.

Effect of concentration of drug on the enrichment %R: With the increase of concentration of drug in feed both Er, %R decrease, for example, in Tables 1 and 2 drug concentration was varied from 0.2-0.6 mM, Er and R values were 34.75, 69.55; 12.63, 46.80 and 40.15, 80.32; 13.08, 48.85, respectively at the pH 5, SGV- 0.054 cm/s. Therefore, it was concluded that separation, enrichment and recovery of drug from dilute feed can be done successfully at low cost by marinating other operating conditions such as optimum pH of feed, optimum concentration of surface active agent (less than CMC), lowest possible velocity of gas that maintains uniform flow of drug foam.

Conclusion

This work presented experimental results of gentamycin sulphate (GEN) separation from its dilute solution using foam separation technique using DOSS and SLS as collector surfactant. The effect of concentration of surface active agent, pH of solution, superficial gas velocity (SGV) and concentration of drug are significant parameters on separation procedure. Results obtained from the experiment indicate that separation, enrichment and recovery of gentamycin sulphate (GEN) from dilute solution can be done successfully at low cost in view of economic operational conditions.

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