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Separation of Less Satisfactory Surface Active Antibiotic (Penicillin G) From Dilute Solution Using Foam Separation Method

GOUTAM MUKHOPADHYAY* and JASMINA KHANAM†
B.C.D.A. College of Pharmacy & Technology, Hridaypur, Barasat, Kolkata-700 127, India
E-mail: goutam_bst@yahoo.com

Penicillin-G is the drug of choice for infection caused by organisms suspectible to it. In case of streptococcal infection (like pharyngitis, scarlet fever), staphylococcus infection, pneumococcus infection, diphtheria, tetanus and gas gangrene, anthrax, actinomycosis and rat bite fever penicillin-G is widely used. Separation and purification of penicillin-G from a mixture of other components is common and essential step in pharmaceutical industry. 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 can be carried out through foam separation method. The aim of this work is to study performance criteria of separation of penicillin-G from an aqueous solution by controlling of different variables and using two type of surfactants in foam separation method. The result showed that penicillin-G can be easily separated from dilute solution of drug mixture in a short time. The influence of operatives 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 follows: concentration of surface active agent tetradecyl trimethyl ammonium bromide (2.25 mM) and dodecylamine (2.25 mM), pH 7, superficial gas velocity 0.03 cm/s, % of drug recovery was found as 84.20 and 86.78, respectively. The unique advantage of the present work relative to other reported method is the higher separation efficiency at lowest cost.

Key Words: Foam separation, Penicillin-G, Surface active agent, Tetradecyl trimethyl ammonium bromide, Dodecylamine.

INTRODUCTION

Adsorptive bubble separation methods (ABSM) is among the less familiar separa-tion methods. The principle of ABSM is based on differences in surface properties of the materials to be separated. Foam separation is a separation method belonging to the ABSM[1]. In foam fractionation, dissolved material is selectively adsorbed on the surface of rising bubbles and then is partially segregated by the

†Pharmaceutical Technology, Jadavpur University, Kolkata-700 032, India.

foam. This fundamental approach stimulated by a great recognition of the potentialities has become an attractive replacement to more costly separation techniques[2-4]. 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[5].

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 for 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[6]. Physico-chemical properties of the materials determine the materials capacity to be separated by foam fractionation. The right choice of columns and auxiliary devices used, together with operational conditions, are of importance to achieve an optimized enrichment. Drugs containing the surface activity under investigation and on the foam fractionation. The equilibrium adsorption of a dissolved material at the gas-liquid interface is given by Gibbs equation:

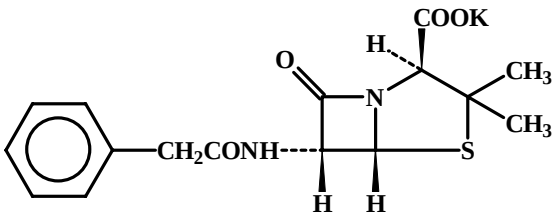
$$d\gamma = - RT \Sigma\Gamma i \, d \ln ai \quad (1)$$

where γ = surface tension; R = gas constant; T = absolute temperature; i = surface concentration of the component i; ai = activity of the component i.

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

where Γ = surface concentration of surfactant; C_B = concentration of surfactant in the bulk. In case of dilute solution ai $\cong C_B$.

Penicillin G ($C_{16}H_{17}KN_2O_4S$) is an anionic β-lactam antibiotic, interfere with the synthesis of bacterial cell wall. It is highly soluble in water and its pH varies between 5.5 and 7.5 determined 10.0 % (w/v) solution. Penicillin G is easily separated by foam separation method with the help of collectors like tetradecyl trimethyl ammonium bromide (TTAB) and dodecylamine (DA). Its activity is limited to gram positive bacteria. *Streptococci aureus* is highly sensitive, but it has now acquired resistance to such an extent that it must be counted out of penicillin G spetrum. Majority of gram negative bacteria and viruses are totally insensitive to penicillin G, except *E. coli*.



Structure of penicillin G

EXPERIMENTAL

Penicillin-G was a gift sample from Standard Pharmaceutical Pvt. Ltd. Howrah. Tetradecyl trimethylammonium bromide (TTAB), dodecylamine (DA) and nile blue indicator was purchased from E. Merck (India) Limited. Foam separation glass column was fabricated by local glass blower. Stalagmometer was purchased from local manufacturer and Shimazdu-1700 UV/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 penicillin G, were purchased from E. Merck (India) Limited.

Glass column with an internal diameter of 4.2 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 feed 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, tetradecyl trimethylammonium bromide (TTAB) and dodecylamine (DA) form stable foam and drug was adsorbed at 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. Then its drug concentration was measured by titremetric assay method or 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.

Titremetric assay of penicillin G (standard solution): First weigh accurately about 0.25 g of the drugs and add 25 mL of water and 25 mL of acetate buffer pH 4.6 and shake until solution is completed. It is titrated immediately at room temperature with 0.02 M mercuric acetate. Each mL of 0.02 M mercuric acetate is equivalent to 0.007450 g of products.

Experimental procedure for separation of surfactant and determination of critical micellar concentration: First step involves the measurement of the critical micellar concentration (CMC) of the surfactants with the help surface tension methods. Then take the known concentration of those surfactant, tradecyl trimethylammonium bromide (TTAB) and dodecylamine (DA) form stable foam through the help of saturated nitrogen 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 was determined by titremetric assay method or UV absorbance using spectrophotometic methods.

Titremetric assay of tetradecyl trimethyl ammonium bromide (TTAB): 100 mL solution was prepared by dissolving 92.51 mg of the TTAB and it was transferred about 25 mL to a separator, add 25 mL of chloroform, 10 mL of 0.1 M sodium hydroxide and 10 mL of freshly prepared 5 % (w/v) potassium iodide. Shake well and discard the chloroform layer. Shake with three quantities chloroform with 10 mL and add 40 mL of dil. HCl. Titrate with 0.05 M potassium iodate until the deep brown colour is almost discarded. Each mL of 0.05 M potassium iodate is equivalent to 0.03364 g of cetrimide ($C_{17}H_{38}BrN$).

Titremetric assay of dodecylamine: 100 mL solution was prepared by dissolving 100 mg of dodecylamine and titrate with 0.1 M perchloric acid using nile blue A as indicator. Each mL of 0.1 M perchloric acid is equivalent to 0.018536 g of dodecylamine.

Sample solution: It is 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. Solvent was added to make up the volume and it was thoroughly mixed.

Tetra-*n*-butylammonium iodide solution: It is transferred 1.250 g of tetra-*n*-butylammonium iodide to a 500 mL volumetric flask, dilute to volume with water and mixed.

Salt solution: 100 g of anhydrous sodium sulfate dissolved and 10 g sodium carbonate in sufficient water to make 1000 mL.

RESULTS AND DISCUSSION

The present work deals with the separation and removal of some medicinal agents such as penicillin G with the help of surface active agents such as tetramethyl trimethyl ammonium bromide (TTAB, m.w. 336.4, CMC 3 Mm) and dodecylamine (DA, m.w. 185.36, CMC 10 Mm), by foam separation method. Surface active agents (SA) 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 colligent. Since these drugs are surface active but foam is not stable, so it can removed/ recovered from dilute solution with the help of collector subs-tances *i.e.*, surface active agents.

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

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

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 recovery: It was observed from Tables 1 and 2, and Figs. 1 and 2 (drug/SA; PG/ TTAB, PG/DA,) that both Er, R values increased with the increase of concentration of SA in feed but decrease when concentration of SA was increased to 2.5 mM at a

TABLE-1
PERFORMANCE CRITERIA OF SEPARATION OF PENICILLIN G WITH TTAB 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 of FD, FM, RS	SGV (cm s^{-1})	Ci (mM)	Cr (mM)	Cf (mM)	Er	Sr	% R
25.5	1/2	37.23, 27.14, 5.20	4, 3.96, 3.50	0.03	1.0	0.142	36.44	36.44	256.6	72.89
25.5	1/2	37.23, 18.81, 5.96	4, 3.96, 3.41	0.04	1.0	0.163	25.32	25.32	155.3	50.65
25.8	1 /2.25	37.23, 32.31, 4.68	7, 6.9, 6.45	0.03	1.0	0.128	43.38	43.38	238.9	86.78
25.8	1 /2.25	37.23, 19.80, 3.41	4, 3.96, 3.41	0.04	1.0	0.150	26.58	26.58	177.2	53.18
25.6	1 /2.5	37.23, 15.59, 5.21	4, 3.91, 3.50	0.03	1.5	0.115	20.79	20.79	180.7	41.60
25.6	1 /2.5	37.23, 13.70, 4.20	4, 3.96, 3.41	0.04	1.5	0.115	18.39	18.39	159.9	36.79
25.6	1.5/2	55.85, 39.09, 11.25	4, 3.96, 3.60	0.03	1.5	0.308	47.71	31.80	154.9	70.00
25.6	1.5/2	55.85, 27.08, 10.15	4, 3.94, 3.61	0.04	1.5	0.278	34.62	23.51	124.5	48.50
25.6	1.5/2.25	55.85, 40.21, 12.40	4, 3.91, 3.40	0.03	1.5	0.339	53.99	35.99	159.2	71.03
25.6	1.5/2.25	55.85, 39.81, 12.00	4, 3.95, 3.50	0.04	1.5	0.331	38.19	25.46	115.3	50.12
25.5	1.5/2.5	55.85, 22.35, 14.90	4, 3.96, 3.41	0.03	2.0	0.408	30.00	20.00	73.52	40.03
25.5	1.5/2.5	55.85, 19.82, 14.00	4, 3.91, 3.41	0.04	2.0	0.383	26.61	17.74	69.47	35.50
25.8	2/2	74.47, 31.70, 18.78	4, 3.91, 3.45	0.03	2.0	0.517	32.74	16.34	63.32	42.56
25.8	2/2	74.47, 24.32, 18.39	4, 3.95, 3.51	0.04	2.0	0.509	21.76	10.88	42.75	32.65
25.9	2/2.25	74.47, 45.68, 21.80	4, 3.96, 3.50	0.03	2.0	0.624	61.33	30.66	98.28	61.34
25.9	2/2.25	74.47, 37.16, 21.59	4, 3.98, 3.41	0.04	2.0	0.589	58.70	29.35	99.66	49.90
26.0	2/2.5	74.47, 28.61, 16.90	4, 3.96, 3.50	0.03	2.0	0.464	34.92	17.46	75.25	38.42
26.0	2/2.5	74.47, 24.72, 18.25	4, 3.98, 3.41	0.04	2.0	0.500	31.61	15.80	63.22	33.20

TABLE-2
PERFORMANCE CRITERIA OF SEPARATION OF PENICILLIN G WITH DA 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 of FD, FM, RS	SGV (cm s^{-1})	Ci (mM)	Cr (mM)	Cf (mM)	Er	Sr	% R
24.5	1/2	37.23, 26.14, 5.20	4, 3.9, 8, 3.70	0.03	1.0	0.142	35.09	35.09	247.1	70.21
24.5	1/2	37.23, 17.87, 5.96	4.3, 95, 3.75	0.04	1.0	0.163	23.99	23.99	147.1	47.99
24.5	1/2.25	37.23, 31.35, 56.67	7, 6.9, 6.5	0.03	1.0	0.182	42.09	42.09	231.2	84.20
24.5	1/2.25	37.23, 23.08, 5.96	4, 3.97, 3.70	0.04	1.0	1.163	26.00	26.00	159.5	62.00
24.5	1/2.5	37.23, 14.89, 4.26	4, 3.50, 3.00	0.03	1.0	0.116	19.99	19.99	172.3	39.99
24.5	1/2.5	37.23, 12.66, 4.48	4, 3.96, 3.00	0.04	1.0	0.122	16.99	16.99	139.2	34.00
24.8	1.5/2	55.85, 36.58, 11.19	4, 3.96, 3.00	0.03	1.5	0.309	36.38	24.25	117.7	65.50
24.8	1.5/2	55.85, 26.31, 9.34	4, 3.5, 3.0	0.04	1.5	0.258	33.64	22.42	130.3	47.23
24.7	1.5/2.25	55.85, 37.98, 11.47	4, 3.5, 3.0	0.03	1.5	0.314	51.00	34.00	162.4	68.00
24.7	1.5/2.25	55.85, 23.79, 10.13	4, 3.3, 2.91	0.04	1.5	0.279	38.30	24.20	137.2	60.50
24.9	1.5/2.5	55.85, 21.72, 14.65	4, 3.3, 2.98	0.03	1.5	0.401	29.16	19.44	72.71	38.90
24.9	1.5/2.5	55.85, 18.54, 13.31	4, 3.91, 3.50	0.04	1.5	0.368	17.16	11.44	46.63	38.20
24.8	2/2	74.47, 29.78, 13.77	4, 3.91, 3.50	0.03	2.0	0.517	30.75	15.37	59.47	40.00
24.8	2/2	74.47, 72.32, 18.39	4, 3.9, 3.6	0.04	2.0	0.509	19.97	9.98	39.23	29.98
25.0	2/2.25	74.47, 44.68, 21.88	4, 3.89, 3.51	0.03	2.0	0.599	59.99	33.50	100.1	60.00
25.0	2/2.25	74.47, 35.37, 19.86	4, 3.96, 3.50	0.04	2.0	0.544	59.36	29.68	109.1	47.50
24.5	2/2.5	74.47, 27.55, 15.96	4, 3.91, 3.42	0.03	2.0	0.441	32.62	16.81	77.82	37.00
24.5	2/2.5	74.47, 23.97, 17.15	4, 3.91, 3.44	0.04	2.0	0.474	25.74	12.87	54.83	32.20

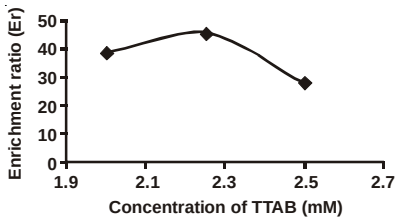


Fig. 1. Effect of concentration of SA (TTAB) on Er values of DG (penicillin G) at 36 ºC

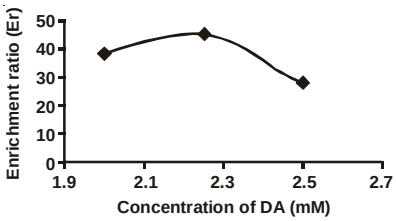


Fig. 2. Effect of concentration of SA (DA) on Er values of DG (penicillin G) at 36 ºC

particular experimental condition (fixed values of pH, SG V and Ci). At this optimum concentration of DA (2.25 Mm), the values Er, Sr, R of drug (penicillin G) were respectively 42.1, 231.26 and 84.20 at pH 7, Ci of drug 1 mM, at lowest SGV value. Similarly at optimum concentration of TTAB (2.25 Mm), values of Er, Sr, R of PG were obtained as 43.38, 238.9, 86.78, respectively. These are slightly higher than DA. Highest enrichment was found incase of drug penicillin G with collector DA at pH 7, SGV-0.03 cm/s and Ci 1 mM.

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 obtained at pH 7. The maximum enrichment and % R at lowest feed concentration (Ci of drug), lowest SGV values, at optimum pH value (7).

Effect of SGV on the % removal and enrichment of drug: Gas flow rate was kept constant at a particular experimental condition by a air flow meter. The were fixed at 0.03, 0.04 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 1-2 mM, Er and R values were 15.37, 40.00; 42.09, 84.20 and 16.37, 42.56; 43.38, 86.78, respectively at the pH 7, SGV 0.03 cm/s. Therefore, it was concluded that separation, enrichment and recovery of drug from dilute feed can be done successfully at low cost by compling other operating conditions such as optimum pH of feed, optimum concentration of SA (less than CMC), lowest possible velocity of gas that maintains uniform flow of foam.

Conclusion

This work presented experimental results of penicillin G separation from its dilute solution by foam separation technique using TTAB and DA 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 drug (penicillin G) from dilute solution can be done satisfactory with simple apparatus without any electricity cost.

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REFERENCES

1. R. Lemlich, Absorptive Bubble Separation Technique, Academic Press, New York (1972).
2. D.W. Armstrong, E.Y. Zhou and S. Chem, *Anal. Chem.*, **64**, 4278 (1998).
3. A.K. Brown and A. Kaul, *J. Biotechnol. Biochem. Eng.*, **62**, 291 (1998).
4. J. Rubio, M.L. Sauja and R.W. Smith, *Mineral Eng.*, **15**, 139 (2002).
5. R.C. Darton, S. Supino and K.J. Sweeting, *J. Chem. Eng. Proces.*, **43**, 477 (2003).
6. P. Ekici and H. Parlar, *Int. J. Food Sci. Nutr.*, **56**, 223 (2005).

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