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Recovery of actinorhodin from fermentation broth

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Abstract

In the present work, a new method of purification for actinorhodin was developed using an expanded bed chromatography technique in which antibiotic capture, feedstock clarification, centrifugation, dialysis and concentration are done in one step. The cation-exchanger (P-11) resulted in 26% adsorption and 2% recovery whereas the anion-exchanger (DE-52) resulted in 99% adsorption and 56% recovery of adsorbed antibiotic using methanol buffer and 2 *M* NH_4Cl as eluting agent. Streamline DEAE anion-exchanger, which is especially designed for EBA applications, yields 82% adsorption and 50% elution of actinorhodin fed into the chromatography column directly from the fermentation broth. Isocratic elution resulted in extremely efficient yield compared to linear gradient elution, i.e. 13.5-fold more recovery in the column with an aspect ratio (L:D) of 4. Expansion by 150% of settled bed resulted in the best recovery of actinorhodin among 100 and 200% expansions. A comparison of breakthrough profiles in packed and expanded bed adsorption showed that the performance of the expanded bed is better (by 33%) at allowing more volume of the fermentation broth to pass through the chromatography column.
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Keywords: Expanded bed chromatography; *Streptomyces coelicolor* A3(2); Actinorhodin; Antibiotics

1. Introduction

Numerous procedures have been devised for the classification, identification, screening and separation of antibiotics using various chromatographic techniques [1,2]. Maeda et al. [3] have shown that most antibiotics are adsorbed on weak or strong cation-exchange resins such as Amberlite IRC-50 or Amberlite IR-120. Bartels et al. [4] developed a technology for sorption of the basic antibiotic streptomycin on a cationic ion-exchange resin from whole unfiltered beer; complex processes were used to purify the streptomycin present in the broth. Industrially, streptomycin is obtained with 75–85% overall yield as a sulfate salt of ca. 99% purity [5]. The acidic antibiotic novobiocin is separated from its broth and purified by a series of specially designed and well mixed columns [1], while the separation of immunomycin from unfiltered whole broth is achieved by the fluidized bed technique using a high-density polymeric resin [6].

Streptomyces coelicolor A3(2) produces four antibiotics: methylenomycin, calcium dependent antibiotic, undecylprodigiosin and actinorhodin [7]. The latter, actinorhodin, is an isochromanequinone antibiotic which inhibits the growth of some Gram-positive bacteria and is classified as antimicrobial in therapeutic category. The recovery of this antibiotic in crystalline form involves several successive time consuming steps such as centrifugation, vacuum filtration, precipitation, drying and grinding followed by two consecutive extractions, evaporation and vacuum crystallization [7]. Incorporation of a chromatographic technique early in the sequence of purification steps will lead to higher product yields

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and a less expensive purification process [8,9]. In the present work, a new method of actinorhodin purification is developed using expanded bed chromatography, in which antibiotic capture, feedstock clarification, centrifugation, dialysis and concentration are completed in a single step. The suitability of ion-exchange adsorbents for actinorhodin separation from the microbial culture is investigated first. Effects of column dimensions, elution mode and buffer type on the adsorption and elution performance of the expanded bed column are studied. Bed expansion and liquid dispersion characteristics, the equilibrium adsorption isotherm and the antibiotic uptake rate are determined experimentally, and the results are compared with correlations given in literature. Comparison of adsorption, washing and elution stages in expanded bed and packed bed chromatography is also made under similar experimental conditions.

2. Experimental

2.1. Organism and culture preparation

The *Streptomyces coelicolor* A3(2) was provided by UMIST, UK, and was maintained as a frozen spore suspension in 20% glycerol at −20°C. Innocula for shake flask culture were prepared according to the reported procedure [10]. A loopful of spore culture was spread on a mannitol/soya agar plate and incubated for 10–14 days at 30°C to allow further sporulation. Then, 2 dm^3 shake flask containing 500 cm^3 YEME medium (3 g yeast extract, 5 g peptone, 3 g malt extract, 10 g glucose, 340 g sucrose per dm^3 of distilled water) was inoculated with 1 cm^3 spore suspension from agar plates and incubated for 10 days on a New Brunswick orbital shaker at 30°C and 200 rpm.

2.2. Ion-exchange adsorbents

The ion-exchangers used as adsorbents, P-11 and DE-52, were purchased from Whatman (Maidstone, UK) and Streamline DEAE was obtained from Pharmacia Biotech (Uppsala, Sweden). They were prepared as described previously [11].

2.3. Buffers

The compositions of mobile phases used in columns are as follows:

(i) Phosphate buffer: 50 m*M* K_2HPO_4 and 50 m*M* KH_2PO_4, pH was adjusted to 7.0 with K_2HPO_4.

(ii) Sodium acetate buffer: 50 m*M* $C_2H_3NaO_2 \cdot 3H_2O$, pH was adjusted to 5.5 with acetic acid.

(iii) Tris buffer: 50 m*M* $NH_2C(CH_2OH)_3$, pH was adjusted to 8.0 with 5 *M* HCl.

(iv) Methanol buffer: methanol–distilled water (64:36), pH 7.7.

(v) Ethanol buffer: ethanol–distilled water (70:30), pH 3.5.

(vi) Acetone buffer: acetone–distilled water (70:30), pH 7.2.

(vii) Pyridine buffer: 5% (v/v) pyridine was dissolved in methanol buffer, pH 7.9.

2.4. Determination of equilibrium adsorption isotherms in the presence of cells

Fifty cm^3 sealed shake flasks containing 0–280 mg/dm^3 of actinorhodin in 25 cm^3 of buffer (methanol, phosphate or sodium acetate) and placed in a shaking water bath at 25°C. Aliquots (2 cm^3) of a 1:1 slurry of adsorbent:buffer was added to each of these flasks. Approximately 24 h was allowed for equilibration, and the contents of the flasks were filtered through 0.45 μm Whatman filter papers. The equilibrium concentration of antibiotic in the bulk liquid phase was determined by UV spectrophotometry at 640 nm after the pH of the solution was adjusted to 12 with 2 *M* NaOH. The equilibrium amounts of antibiotic bound per cm^3 of the ion-exchange adsorbent was determined by mass balance.

2.5. Measurement of bed expansion characteristics

DE-52, P-11 or Streamline DEAE was placed in 1.5, 2.5 and 5 cm I.D. columns to give different settled bed heights. The bed was expanded by increasing the flow-rate through the column at 0.5 cm^3/min steps until the desired bed expansion was reached. Twenty minutes were allowed between each step. The variation in bed height was recorded as a function of superficial liquid velocity.

2.6. Expanded bed chromatography experiments

The expanded bed chromatography columns (30 cm×1.5 cm, 10 cm×2.5 cm, 30 cm×2.5 cm and 10 cm×5 cm) and adaptors (I.D. 1.5, 2.5, and 5 cm) were purchased from Bio-Rad (Hercules, CA, USA). The adaptor that was used to minimize the volume of liquid above the expanded bed had a variable working length of 1–14 cm. The Model EP-1 Econo Pump is a two-channel, bi-directional, variable speed peristaltic pump from Bio-Rad. The columns were packed with the relevant ion-exchange adsorbents, which were fluidized and equilibrated with buffer solution to give a stable expanded bed height prior to adsorption studies. The fermentation broth antibiotic was placed in a reservoir and pumped by a peristaltic pump upwards through the column. The column was then washed with the buffer, with monitoring by UV absorbance, to remove the unbound components. The superficial velocity at the adsorption and wash stages was progressively changed to maintain a constant degree of bed expansion. The bound antibiotic was recovered using either isocratic or linear gradient elution methods. In isocratic elution, the elution mode was downward, using 2 *M* NaCl or 2 *M* NH_4Cl in relevant buffer solution as the eluting agents. In gradient elution, a linear gradient of the eluting agent (0–2 *M*) in buffer was flown upward. The presence of the antibiotic, actinorhodin, was determined in the feedstock, flowthrough, wash and eluate.

2.7. Packed bed experiments

The up-flow packed bed experimental setup (column 4 cm×2.5 cm) is the same as those used in the expanded bed experiments. The dead volume at the top was minimized. The superficial velocity was kept constant at 32 and 25 cm/h in the adsorption and elution stages, respectively.

2.8. Antibiotic assay

Actinorhodin concentration is determined as described previously [7,10]. Actinorhodin was extracted from a known volume of sample by adding an equal volume of 2 *M* NaOH. Then the solution was adjusted to pH 12 by 2 *M* HCl. After centrifuging at 4000 rpm for 10 min to remove any suspending solids, the absorbance of the supernatant was measured at 640 nm by a UV–Vis spectrophotometer against media blanks. Actinorhodin concentration was calculated by using the extinction coefficient, $E^{1\%,}_{1\ \mathrm{cm}}=355$.

3. Results and discussion

3.1. Bed expansion characteristics

Bed expansion characteristics are known to depend on a number of variables, such as the viscosity and the density of the process liquids and the certain physical properties of the adsorbent beads (i.e., distribution of size and density) [12]. The variation in the degree of expansion of DE-52 adsorbent bed as a function of superficial velocity of liquid flow through the bed was measured for two different buffers and column diameters (1.5 and 2.5 cm).

Richardson–Zaki correlation of the form $u_0 = u_t(\epsilon)^n$ was used to predict the terminal velocity of adsorbents; where u_0 is the superficial liquid flow velocity, u_t is the Stokes' settling velocity of particles at infinite dilution, and n is the Richardson–Zaki exponent. The void fraction ϵ is calculated as:

$$\frac{H}{H_0} = \frac{1-\epsilon_{\min}}{1-\epsilon}$$

where $\epsilon_{\min}$ is the minimum bed void fraction and is approximately 0.42 for a settled bed of spherical particles [6].

The Richardson–Zaki parameter, n, and the settling velocity of particles, u_t, are found from linear regression of plots of ln u versus ln ϵ determined at two different column diameters (Table 1). The linear regression coefficients, r, are calculated as 0.99 for both of the column diameters. The experimentally determined values of the Richardson–Zaki parameter, n, are close to the theoretical value of 4.8, which is normally used in the laminar flow regime [12]. The values of n in Table 1 show a variation of 3–5% across the range of results obtained and thus, the bed was assumed to be stable upon expansion.

The u_t values predicted by Stokes' equation range from 72 to 292 cm/h, because of the size distribution

Table 1
Physical properties and Richarson–Zaki parameters

Buffer solution	Column diameter (cm)	Solution density (kg/m^3)	Solution viscosity (cp)	Settled bed height (cm)	u_t Exp. (cm/h)	u_t (Stokes) (cm/h)	n
Methanol	1.5	856.6	0.776	8	122	72–292	4.67
Phosphate	2.5	1000	1	8	110	33–130	4.60

of DE-52 adsorbent particles between 30 and 60 μm. The experimental settling velocities, u_t, determined by Richardson–Zaki correlation are in the range of Stokes' settling velocities. It was reported that if the theoretical settling velocities were less than the calculated experimental values, the physical properties of adsorbent beads would be affected by the presence of cells, causing them to agglomerate [13]. However, we demonstrated that the bed expansion characteristics of conventional DE-52 adsorbents obey the Richardson–Zaki correlation with the terminal velocity of adsorbent beads being satisfactorily predicted from the Stokes' equation.

Significant wall effects on expanded bed stability were reported when the ratio of the column diameter to adsorbent bead diameter is less than 10 [13]. In the present work, we observed that this effect could be neglected even if the ratio was up to 800 using DE-52 bead as adsorbent.

3.2. Expanded bed experiments

This paper presents the first results for the direct extraction of actinorhodin from the Streptomyces broth in a single step without prior clarification/purification. The production of antibiotic actinorhodin by *Streptomyces coelicolor* A3(2) strain was found to increase in YEME medium containing complex nutrients. A high amount of sucrose (340 g/dm^3) was added into the fermentation system for osmotic balance of the culture.

The solid content of the fermentation broth was measured to be about 13% (w/w). Feedstock with a biomass dry mass higher than 8% was reported to cause channeling in the expanded bed and poor recovery of the target bioproduct [14]. Therefore, when necessary, the stock fermentation broth was diluted in 1:2 ratio with methanol buffer before being pumped into the expanded bed in order to prevent colloidal material from being trapped in the column.

The fermentation broth loading was always kept below 20% of the estimated dynamic capacity of the column to avoid saturation with actinorhodin and any loss of yield due to the breakthrough of unadsorbed antibiotic. The ratio of feedstock volume to the settled absorbent volume was about 2–7 depending on the initial concentration of actinorhodin in broth. The operational flow-rates were between 36 and 200 cm/h. When conventional adsorbents, DE-52 and P-11, were used, 2–3-fold expansion could be obtained in the lower flow-rate range. Operational flow-rates in the higher range of 100–200 cm/h were also used to diminish the mass transfer resistance of the purified bioproduct at absorbent surface and the processing time, thus improving the system productivity [15].

3.2.1. Adsorption stage

Intact whole cells and disrupted cells passed freely through the expanded bed. Since the height of the expanded bed dropped due to adsorption, the flow-rate through the bed was increased gradually in order to maintain a constant degree of bed expansion. The expanded bed height at any time could be observed even in turbid feedstock since the antibiotic actinorhodin is pigmented.

The dynamic capacity of the bed was determined when the actinorhodin concentration in the column effluent reached 10% of the initial concentration. The dynamic capacity ($Q_T = C_o \cdot V_b / V_{settled}$) of DE-52 was calculated as 1.54 mg actinorhodin/cm^3 DE-52 adsorbent bed at 10% of breakthrough. Nigam et al. [16] observed by scanning electron microscopy that cells and cell debris adhere to the positively charged surface of DEAE–trisacryl adsorbent. In the present work, cells/cell debris and other colloidal material

such as unconsumed substrates do not seem to affect the binding of actinorhodin.

3.2.2. Washing stage

The unbound substances were removed from the column using 2–5-fold bed volumes of buffer. The superficial velocity was increased from 34 to 102 cm/h to maintain a constant degree of bed expansion. The loss of actinorhodin during the flowthrough and wash stages was found to be negligible.

3.2.3. Elution stage

Elution of actinorhodin was first performed in down-flow packed bed mode. Depending on column geometry, the superficial velocity was selected between 66 and 198 cm/h. The eluted fractions changed color gradually from dark red to colorless/transparent. Particulate material was not observed in the eluate. The adsorbed actinorhodin was eluted as a relatively sharp peak. A 2–3-fold enriched antibiotic fraction is obtained by 5–10-fold bed volumes of solution. It is reported that up to 3–5-fold purification can be obtained by expanded bed chromatography in the presence of cells [17].

Elution yield was expressed as the percentage of the total amount of actinorhodin in eluate per amount of actinorhodin adsorbed onto the ion-exchange matrix. At most, 61% of the adsorbed actinorhodin was recovered and the maximum peak showed a purification factor of 5.

Elution in packed mode with a downward flow has so far been a standard method for the recovery of adsorbed material in order to maximize product concentration and minimize consumption of elution buffer. However, the pressure stability of the matrix during repeated use is reported to be as the main drawback of the packed bed elution [18]. Therefore, elution was also tested in expanded bed mode gradient-wise. In linear gradient elution, the eluting agent (0–2 *M* NH_4Cl or 0–2 *M* NaCl) in an appropriate buffer was fed into the column at a superficial velocity of 25 cm/h in 45 min or at 68 cm/h in 100 min in expanded bed mode where the bed expansion was kept constant similar to the adsorption and washing stages. Elution yields were in the range of 2.6–10.9% of actinorhodin adsorbed onto the column. The peak shapes were mostly irregular for expanded bed elution experiments.

When both elution techniques at the same linear flow-rate of 68 cm/h were compared, 61.2% actinorhodin recovery was obtained with packed bed mode and 10.9% with gradient elution and expanded bed mode. The experimental data indicate that actinorhodin is adsorbed on the surface of the ion-exchanger and is also able to access the interior of the cellulosic ion-exchange matrix which results in an asymmetric elution curve with tailing.

A multistage stepwise elution protocol was also tested using 1, 2 and 3 *M* NaCl eluting agents in buffer, resulting in 35, 61, and 15.2% elution yields, respectively.

In the present work, one cycle of purification process including equilibration (40 min) + loading (30–50 min) + wash (20–50 min) + elution (50 min) took 3–4 h. Productivities [12,19] for actinorhodin are found to be in the range of $1–8 \cdot 10^{-3}$ mg actinorhodin per cm^3 of adsorbent per min.

3.3. Choice of ion-exchange matrix

In the development of a purification process for actinorhodin, the first step is the choice of the most appropriate ion-exchange adsorbent. Three different ion-exchange matrices, i.e. cation-exchanger P-11, anion-exchanger DE-52 and purpose-designed anion-exchanger Streamline DEAE were used in this study. The other matrix, Streamline Q, was reported to interact strongly with unbroken cells/cell debris, and therefore, is not considered for the extraction of bioproducts from feedstocks [19].

The cation-exchanger P-11 was tested using 50 m*M* sodium acetate buffer at pH 5.5 in a 2.5-cm I.D. column; the settled and expanded bed heights were 4 and 10 cm, respectively. The fermentation broth containing 5.1 mg actinorhodin was loaded onto the column of which 43.8% was adsorbed and 8.83% was eluted. The dynamic capacity of P-11 was calculated as 0.18 mg actinorhodin/cm^3 P-11 adsorbent bed at 10% of breakthrough. These results indicated that P-11, with its orthophosphate functional group and ammonium counter ion, is not suitable for actinorhodin purification.

3.3.1. Comparison of anion-exchangers

DE-52 and Streamline DEAE were tested in a 1.5-cm I.D. and 30-cm height column using metha-

nol–water buffer (64:36). Fermentation broth with 15.2 mg actinorhodin was fed into each column. The settled and expanded bed heights in both columns were 8 and 20 cm, respectively. Experimental data are compared in Fig. 1.

The type of anion-exchanger used affected the peak position and shape of the adsorption curves. The adsorption curve of DE-52 indicates that 10% breakthrough is reached at a later time, i.e. 80 cm^3 of fermentation broth can be fed onto the DE-52 column as compared to the 25 cm^3 loading on the Streamline DEAE column. Adsorption yields are 90.8% of actinorhodin in feedstock for DE-52 and 82.2% for Streamline DEAE.

Zhang et al. [15] reported that the denser the adsorbent particle, the lower the physical and biochemical capacity for bioproduct. The equivalent diameters of the DE-52 particles are between 30 and 60 μm, while those of Streamline DEAE are between 100 and 300 μm. The dynamic binding capacity of the adsorbents were determined at 10% of breakthrough as 1.54 and 0.71 mg actinorhodin per cm^3 of adsorbent for DE-52 and Streamline DEAE, respectively, which is consistent with Zhang's proposal.

Since the density of Streamline DEAE particles (1200 kg/m^3) is higher than that of DE-52, the flow-rate to expand is also higher. The superficial velocity in the 1.5-cm I.D. column with Streamline DEAE was 2.2 times greater than that of DE-52, and 16.6 times greater in the 5-cm I.D. column at 150% bed expansion. The higher flow-rate would be expected to help actinorhodin molecules overcome the film resistance on the adsorbent surface, while at the same time reducing the contact time for interaction between adsorbent and antibiotic. Consequently, DE-52 anion-exchanger gives a better result compared to Streamline DEAE in the adsorption stage.

Elution behaviour of Streamline DEAE was found to be similar in shape to that of DE-52. However, the yield is 55.8% for DE-52 and 49.8% for Streamline DEAE, indicating that DE-52 is also better in the elution stage.

The equilibrium adsorption isotherm on the ion-exchange matrix as DE-52 was determined by plotting the antibiotic adsorbed per volume of DE-52 versus the equilibrium concentration of antibiotic in methanol buffer. Although antibiotic adsorption occurred simultaneously with adsorption of cells to the adsorbent, the adsorption isotherm for actinorhodin could be described by a Langmuir type adsorption isotherm (Fig. 2). Such behavior might not be expected in multi-component adsorption, but can be explained by the very small degree of adsorption of cells present. The maximum equilibrium binding capacity, q_{max}, is calculated as 3.50 mg actinorhodin/cm^3 adsorbent, and the dissociation constant, K_D, is 3.64 mg actinorhodin/cm^3 solution.

3.4. Choice of buffer

The buffers used in expanded bed experiments are summarized in Table 2. In all these experiments, the amount of actinorhodin in the fermentation broth loaded into the columns was the same, i.e. 15 mg.

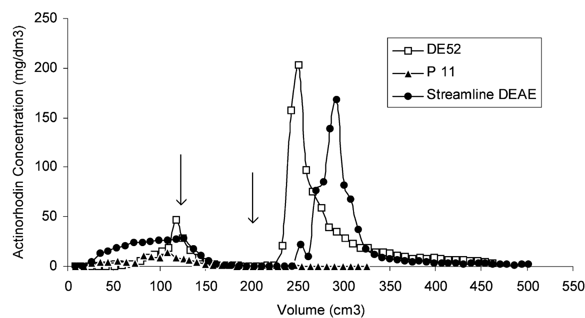

Fig. 1. Comparison of expanded bed chromatography experiments using different ion-exchange adsorbents (Arrows indicate the start of wash and elution stages).

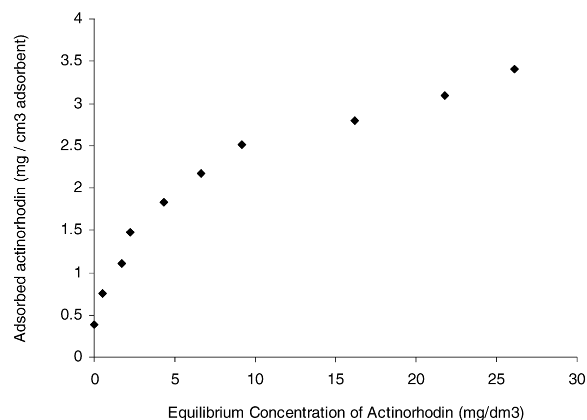

Fig. 2. Equilibrium Adsorption Isotherm for Actinorhodin.

Table 2
Comparison of the buffer systems

Buffer	Column height×diameter (cm×cm)	SBH (cm)-EBH (cm)[a]	pH of the buffer	Dynamic binding capacity (mg/cm^3)	Adsorption yield (%)	Elution yield (%)
Methanol	30×1.5	8–20	7.7	1.54	91	56
Phosphate	30×2.5	4–10	7.0	0.25	95	29
Sodium acetate	30×1.5	14–30	5.5	0.61	94	26
Acetone	30×1.5	8–20	7.2	0.72	82	53
Ethanol	30×1.5	8–20	3.5	0.85	52	49
Tris	30×1.5	8–20	8.0	1.31	88	21
Pyridine	30×2.5	8–20	7.9	0.58	100	44

[a] SBH=Settled bed height; EBH=expanded bed height.

Each column contained 14.1 cm^3 adsorbent except the experiments with phosphate and sodium acetate buffers, where the columns contained 19.6 and 24.5 cm^3 of adsorbents, respectively.

The buffers used in the adsorption, washing and elution stages are shown in Fig. 3 and the results are summarized in Table 2. The feedstock was loaded onto the columns until the level of actinorhodin in the flowthrough had risen to about 10% of breakthrough. When the dynamic capacities of the expanded bed with different buffers are compared, methanol buffer (pH 7.7) was found to result in the highest capacity. Although the adsorptive capacity of the anion-exchange adsorbent in Tris buffer was found to be close to that obtained in methanol buffer, its elution yield is very low, i.e. 21%. In industry, methanol buffer is a preferred solvent due to its low cost and rapid biodegradability [6]. A minimum ratio of 1:2 methanol/feedstock was reported to dissociate the product from the cells [6]. Therefore, when methanol buffer was used, the feedstock was diluted in a 1:2 ratio, which helped to dilute the highly viscous feedstock and thus, prevent the blockage of the column.

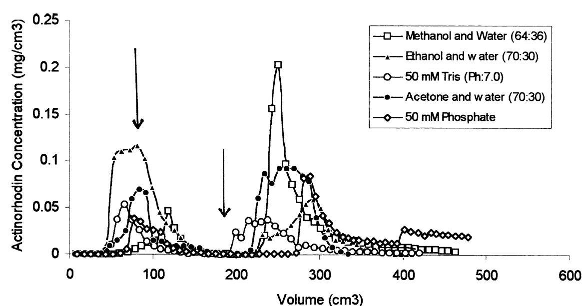

Fig. 3. Comparison of different buffers used in expanded bed chromatography experiments (Arrows indicate the start of wash and elution stages).

The most important factor in the choice of buffer solution depends on the pH and ionic strength to be used. The maximum solubility of actinorhodin is achieved around pH 7.5. When the pH of the buffers are compared, pH of 7.7 belongs to the methanol buffer system which gives the highest adsorption yield among the columns having the same volume of adsorbent. The conductivity of the methanol buffer with the eluting agent of 2 *M* NH_4Cl was found to be relatively higher than the conductivity of 50 m*M* Tris buffer with 2 *M* NaCl which supports the observation that actinorhodin cannot desorb easily during the elution stage in Tris buffer. The conductivity of the solution needs to be high to desorb the bioproduct [14].

3.5. Column diameter

The objective of these experiments is to find the best column geometry for actinorhodin purification. The anion-exchange columns of 30 cm×1.5 cm and 10 cm×2.5 cm were compared using the same volume of DE-52 adsorbents and methanol buffer. The fermentation broths loaded onto the columns contained 15.1 mg actinorhodin. Table 3 summarizes the experimental conditions and results.

Earlier elution of actinorhodin in highest bed expansion (150–170%) and relatively late elution of actinorhodin in lowest bed expansion (70–80%) were obtained in both column geometries. In these experiments, the 1.5-cm I.D. column results in better

Table 3
Comparison of anion-exchange columns of 1.5, 2.5 and 5 cm internal diameter

Column (height×diameter) (cm)	Aspect ratio (length/diameter)	Adsorbent volume (cm^3)	SBH (cm)-EBH (cm)	Bed expansion (%)	Adsorption yield (%)	Elution yield (%)
30×1.5	20	19.7	11–30	170	97.6	47.5
		24.6	14–30	120	99.2	51.6
		29.2	16.5–30	80	99.6	53.0
10×2.5	4	19.7	4–10	150	98.6	37.0
		24.6	5–10	100	95.2	31.1
		29.2	6–10	67	97.9	34.2
10×5	2	39.2	2–5	150	93.9	25.2
30×2.5	12	39.2	8–20	150	89.6	42.2

adsorption and elution yields. These experiments demonstrate that adsorption and elution yields increase while the column diameter decreases. When the aspect ratio of the columns are compared, the column with the biggest aspect ratio gives best results in terms of adsorption and elution yields. The adsorption and elution yields decreased with decreasing aspect ratio. A further comparison between the 5- and 2.5-cm I.D. columns using methanol buffer system again showed that the recovery of the experiment with the 5-cm I.D. column is lower than the recovery of experiment in the 2.5-cm I.D. column due to smaller aspect ratio.

3.6. Effect of settled bed height and expanded bed height

The adsorption of actinorhodin from the fermentation broth was carried out at different degrees of bed expansion but always using the same volume of adsorbent (14 cm^3) in the 1.5-cm I.D. column and methanol buffer. Experimental conditions and results are given in Table 4.

The adsorption yields are similar, i.e. 91–93% of loaded actinorhodin with an ion-exchange volume of 14 cm^3. An effective expanded bed operation is achieved when the volume of expanded bed is 1.5 times the volume of settled bed. The actinorhodin was eluted with 10 bed volumes of buffer containing 2 *M* NH_4Cl. Elution volume is defined as the volume obtained between the beginning of the elution peak and the end point where 0.05 mg actinorhodin in a fraction (0.3% of loaded actinorhodin) is eluted.

The antibiotic, actinorhodin, appeared earlier in the flow through in the experiment with 100% expansion (16 cm expanded bed height). Moreover, 100% bed expansion allowed about 70 cm^3 of fermentation broth to be effectively processed whereas 150 and 200% bed expansions allowed more than 100 cm^3 of fermentation broth to pass through the column without any traces of actinorhodin in the effluent stream.

3.7. Packed bed versus expanded bed system

In order to study the relative performance of the

Table 4
Comparison of anion-exchange columns of 1.5 cm internal diameter at different settled bed (SBH) and expanded bed heights (EBH)

SBH (cm)-EBH (cm)	Ion-exchange adsorbent volume (cm^3)	Adsorption stage flow-rate (cm/h)	Adsorption yield (%)	Elution yield (%)	Elution volume (bed volume)	Elution stage flow-rate (cm/h)
8–16	14.1	21	92.8	51.5	7.1	50
8–20	14.1	34	90.8	55.8	10.0	68
8–24	14.1	44	90.5	45.0	5.5	68
11–30	19.7	44	97.6	47.5	6.2	68
14–30	24.6	34	99.2	51.6	3.7	68
16.5–30	29.2	21	99.6	53.0	3.0	68

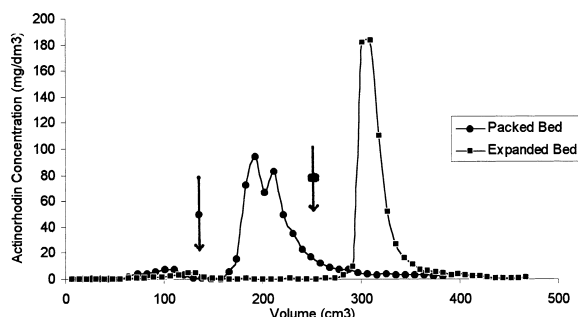

Fig. 4. Comparison of packed and expanded bed chromatography experiments (Arrows indicate the start of elution stages in packed and expanded beds).

bed in the packed and expanded mode operating strategies, experiments were performed using the same amount of adsorbent in a 10 cm×2.5 cm column at the same superficial velocity (Fig. 4). Methanol buffer was used in these experiments and the initial concentration of actinorhodin in fermentation solution was 168 mg/dm^3. Both experiments were performed at 4 cm initial settled bed heights.

As can be seen from Fig. 4, the packed column reached the breakthrough point (5% of feed concentration of actinorhodin) at 120 cm^3 of fermentation broth, whereas the expanded column reached the breakthrough point at 165 cm^3. Thus, 33% more fermentation broth can be processed by expanded bed chromatography. This indicates that the packed bed will be unable to operate for long periods when the particulate material such as cells and cell debris are present. The solid content of the broth was up to 13% and it caused a decrease in the flow-rate of the feedstock because of the clogging.

When the dynamic binding capacities of expanded and packed bed columns were compared, 1.60 and 1.03 mg actinorhodin per cm^3 of DE-52 adsorbent were obtained at 5% breakthrough of actinorhodin, respectively.

4. Conclusion

Direct extraction techniques in bioseparation have a significant impact on the economics of bioproducts which are for use in the diagnostic and health care industries. The work presented here shows that the antibiotic actinorhodin can be purified from unclarified fermentation broth of *Streptomyces coelicolor* A3(2) using ion-exchange expanded bed chromatography. The use of expanded bed chromatography eliminates the clarification step, and produces concentrated and partially purified antibiotic ready for the next purification step. The recovery of actinorhodin on elution from the expanded bed compares well with other results shown in the literature for a less specific method, i.e. ion-exchange chromatography. Ion-exchange adsorbents are preferred for their relative cheapness and they can also be subjected to harsh conditions such as ethanol, methanol, etc. When less specific adsorbents are used, the presence of cells is expected to affect the adsorption of the target bioproduct, and this effect will be greatest at high cell concentrations. The relative non-specificity of ion-exchangers allows for competitive adsorption between the target bioproduct and cells which also have charged surfaces [17]. As an alternative, direct extraction could be carried out with highly specific matrices such as affinity adsorbents.

The development of a mathematical model for the prediction of the rate-controlling mechanisms in adsorption and elution stages of actinorhodin purification is currently in progress. The extension of the modeling to the design of a commercial-scale primary isolation process for actinorhodin has also being undertaken.

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