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# Purification of Forskolin by Adsorptive Separation Using Functionalized Polymer Bearing Specific Ligands Designed by Molecular Simulation

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**ABSTRACT:** The adsorption of forskolin and its analogues has been studied, a priori, by molecular modeling to design a suitable adsorbent for selective adsorption of forskolin from a complex extract. Ligands selective for forskolin have been designed on the basis of two-point attachments with forskolin so as to have desired selectivity. Dynamic adsorption and desorption experiments were conducted to establish the optimum parameters for the purification process using a functionalized polymer. After a single adsorption and desorption cycle, the purity of the forskolin increased to 98% using phenyl glycine-*p*-sulfonic acid substituted polystyrene as a functionalized polymer. Experimental verification of the selectivity of the ligand toward forskolin was successfully achieved.

## INTRODUCTION

Forskolin (colforsin), a diterpenoid (Figure 1) present in the roots and stems of *Coleus forskohlii* Briq, shows a remarkable pharmacological activity for treatment of congestive heart failure, glaucoma, asthma, and certain types of cancer and to promote lean body mass.[1−6]

Apart from forskolin, its analogues 7-deacetylforskolin and 1,9-dideoxyforskolin are also present in the roots as minor constituents (Figure 1). A synthetic route for forskolin has been reported. However, the product was not as effective as that obtained from the natural source.[7] Production of forskolin has been reported in cell cultures, but it is observed that microbial production of forskolin requires 4−5 weeks of fermentation time and forskolin is produced in low yields.[8−11] Extraction of forskolin from coleus roots is usually conducted with organic solvents, supercritical $CO_2$, or aqueous solutions and its subsequent purification by column chromatography and/or repeated crystallization involving multiple solvents is essential.[12−21]

Most of the forskolin purification techniques involve column chromatography on silica gel followed by successive crystallizations involving multiple solvents. Bhat et al.[19] have reported purification of forskolin on a silica column using petroleum ether−benzene, benzene−ethyl acetate, and ethyl acetate−methanol mixtures as the mobile phase. Bhat et al.[20] have also reported another purification process for forskolin wherein the crude forskolin extract was treated with a base to obtain a crude terpenoid mixture before column chromatography and crystallization. Saleem et al.[21] have reported isolation of forskolin from crude extract using activated charcoal as a reverse phase adsorbent wherein the elution was carried out under reduced pressure and the eluate obtained from the column was used for crystallization in different solvent mixtures to obtain pure forskolin. The use of simple and cheaper adsorbents such as silica and charcoal looks attractive, but at the same time cumbersome postcolumn treatments such as crystallizations involving different solvents make the process tedious. The losses of solvent and adsorbent such as silica add to the operating cost. Most of the impurities present in the crude extract of *C. forskholii* are highly polar complex organic materials and are irreversibly adsorbed on silica.

Yanagihara et al.[22] have reported isolation of forskolin from the crude extracts of *C. forskohlii* by immunoaffinity column chromatography using antiforskolin monoclonal antibodies on silica gel column. Only the fraction containing compounds similar to forskolin was subjected to the immunoaffinity separation. However, forskolin was contaminated by 7-deacetylforskolin. Efficient separation of forskolin from its analogues is still a challenging task owing to their similar physical properties. Design of affinity ligands specific for target molecules, particularly for chiral mixtures, has found growing interest in recent years.[23−32]

We have recently reported selective sorption of forskolin analogues on a diethanolamine loaded polystyrene matrix as an adsorbent designed by molecular simulation.[31] As an extension of this approach, we present here a combination of molecular simulation for design of ligands and their subsequent synthesis and loading on a polymer matrix to develop affinity adsorbents selective for forskolin. The emphasis is on the approach of manipulating selectivity of adsorptive separation by understanding the interaction between forskolin and different ligands at molecular levels.

## MOLECULAR SIMULATION OF AN AFFINITY ADSORBENT SELECTIVE FOR FORSKOLIN

Forskolin differs in its structure from its analogues, namely, 1,9-dideoxyforskolin and 7-deacetylforskolin, only in the presence of hydroxyl groups. We initially thought of an affinity adsorbent with maximum affinity toward the hydroxyl groups at the first and ninth positions of forskolin. The carbonyl group can be further considered for multiple-point attachment for the interaction with the ligand. On the basis of this three-point attachment, three different ligands, namely, *N*-propinoyl aspartic acid (NPAA), phenyl glycine-*o*-carboxylic acid (PGOCA), and phenyl glycine-*p*-sulfonic acid (PGPSA), were considered for molecular simulation.

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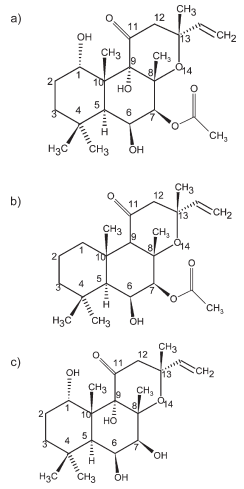

**Figure 1.** Structures of forskolin and its analogues: (a) forskolin; (b) 1,9-dideoxyforskolin; (c) 7-deacetylforskolin.

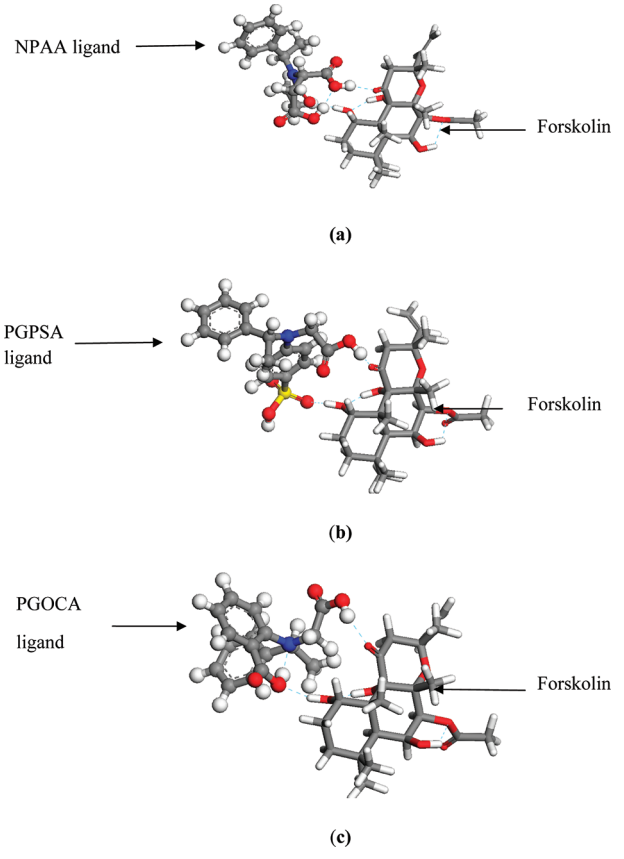


**Figure 2.** Interaction of forskolin with (a) NPAA ligand, (b) PGPSA ligand, and (c) PGOCA ligand. The presence of hydrogen bond is indicated by dotted lines.

The interaction studies of forskolin and its analogues with these ligands were carried out in the same manner as mentioned in our previous report.[31] Initial optimization was performed by molecular mechanics (MM) calculations using the COMPASS force field of Material Studio (version 4.1, Accelerys). The MM optimized structures were further optimized by density functional theory (DFT) calculations of the DMol$^3$ module of Material Studio using the double numerical plus polarization (DNP) basis set that includes a polarization p-function on all hydrogen atoms and has the best accuracy for predicting structural properties and most importantly for hydrogen bonding.[33] The exchange correlation energy was calculated using the generalized gradient corrected functional BLYP correlation.[34] The optimized structure of forskolin showed good agreement with the X-ray crystallographic data in terms of bond distances.[35]

**Table 1. Interaction Energy Values of 1:1 and 1:2[a] Complexes of Forskolin/Analogues with Various Ligands**

| component | interaction energy (kcal/mol) before solvation | after solvation in ACN | after solvation in MeOH |
|---|---|---|---|
| | NPAA Ligand | | |
| forskolin | −11.13 | −81.6 | −132.26 |
| | (−14) | (−189) | (−147) |
| 1,9-dideoxyforskolin | −12 | −75.6 | −126.63 |
| | (−12) | (−155) | (−139) |
| 7-deacetylforskolin | −11 | −76.27 | −122.87 |
| | (−11) | (−176) | (−134) |
| | PGPSA Ligand | | |
| forskolin | −24.76 | −107.96 | −85.10 |
| | (−13.9) | (−137) | (−87) |
| 1,9-dideoxyforskolin | −20.92 | −103.07 | −79.66 |
| | (−13.5) | (−131) | (−79) |
| 7-deacetylforskolin | −24.29 | −108.11 | −87.31 |
| | (−12.7) | (−125) | (−85) |
| | PGOCA Ligand | | |
| forskolin | −24.94 | −144.18 | −126.55 |
| | (−18.6) | (−142) | (−73) |
| 1,9-dideoxyforskolin | −19.37 | −129.11 | −124.57 |
| | (−17.8) | (−124) | (−73) |
| 7-deacetylforskolin | −23.72 | −131.79 | −125.22 |
| | (−17.4) | (−138) | (−58) |

[a] The values in parentheses are for 1:2 complexes.

The molecular simulation shows that the hydroxyl groups at the first and sixth positions of forskolin form intramolecular hydrogen bonds with other groups in their vicinity. Contrary to our initial expectation, the hydroxyl group at the ninth position is not easily available for interaction with any ligand due to steric constraints. The first hydroxyl group and carbonyl group at the 11th position of forskolin are the main interacting centers observed for all three ligands, as shown in Figure 2. In the case of the NPAA ligand, even the ninth hydroxyl group of forskolin interacts with the acidic hydrogen of the ligand though with a greater bond distance (3.6 Å) but is unlikely to form any effective H-bond.

Unlike forskolin, only a single-point interaction was observed between the carbonyl group at the 11th position of 1,9-dideoxyforskolin and acidic hydrogen of all three ligands. In the case of 7-deacetylforskolin, the observed interactions were similar to those of forskolin.

The interaction energy (IE), in a vacuum, was higher for forskolin than those for 1,9-dideoxyforskolin and 7-deacetylforskolin with the ligands, but the difference in the IE is not large, which is not surprising considering their very similar structures (Table 1).

**Table 2. Distances between Interacting Centers of Forskolin and Its Analogues with Various Ligands[a]**

| | bond distance between first OH group and ligand (Å) | | | | bond distance between 11th carbonyl group and ligand (Å) | | | |
|---|---|---|---|---|---|---|---|---|
| | MM value | | | | MM value | | | |
| component | vacuum | ACN | MeOH | DFT value | vacuum | ACN | MeOH | DFT value |
| | | | | NPAA Ligand | | | | |
| forskolin | 1.82 | 1.61 | 2.65 | 2.16 | 1.75 | 1.65 | 1.76 | 1.79 |
| | (2.20) | (1.62) | (2.89) | (2.16) | (1.73) | (1.60) | (1.75) | (1.79) |
| 1,9-dideoxyforskolin | – | – | – | – | 3.87 | 2.83 | 2.36 | 1.77 |
| | | | | | (1.76) | (1.84) | (1.68) | (1.77) |
| 7-deacetylforskolin | 1.88 | 1.82 | 2.17 | 2.05 | 1.74 | 2.16 | 1.82 | 1.76 |
| | (1.8) | (1.81) | (2.00) | (1.95) | (1.74) | (2.02) | (1.80) | (1.76) |
| | | | | PGPSA Ligand | | | | |
| forskolin | 1.76 | 1.56 | 2.35 | 2.21 | 1.89 | 1.68 | 2.13 | 1.88 |
| | (1.66) | (1.50) | (2.06) | (2.21) | (2.38) | (1.67) | (1.60) | (1.88) |
| 1,9-dideoxyforskolin | – | – | – | – | 1.65 | 1.64 | 1.70 | 1.81 |
| | | | | | (1.77) | (1.65) | (1.60) | (1.70) |
| 7-deacetylforskolin | 1.73 | 3.52 | 2.61 | 2.36 | 2.28 | 4.68 | 2.61 | 1.85 |
| | (2.40) | (3.52) | (2.82) | (2.39) | (1.73) | (4.90) | (2.28) | (1.85) |
| | | | | PGOCA Ligand | | | | |
| forskolin | 1.76 | 1.95 | 1.74 | 1.99 | 1.93 | 1.95 | 2.75 | 1.79 |
| | (1.9) | (1.91) | (1.74) | (1.99) | (2.4) | (1.82) | (2.60) | (1.79) |
| 1,9-dideoxyforskolin | – | – | – | – | 1.80 | 1.83 | 3.05 | 1.81 |
| | | | | | (1.80) | (1.87) | (3.20) | (1.80) |
| 7-deacetylforskolin | 1.83 | 2.26 | 2.38 | 1.91 | 1.87 | 3.46 | 2.65 | 1.79 |
| | (1.85) | (2.26) | (2.31) | (1.91) | (1.90) | (3.55) | (2.22) | (1.81) |

[a] The values in parentheses are for 1:2 complexes.

**Table 3. Charges on Interacting Centers**

| component | first hydroxyl hydrogen | 11th carbonyl oxygen |
|---|---|---|
| | *N*-Propinyl Aspartic Acid | |
| forskolin | 0.349 | −0.570 |
| 1,9-dideoxyforskolin | – | −0.561 |
| 7-deacetylforskolin | 0.346 | −0.574 |
| | Phenyl Glycine-*p*-sulfonic Acid | |
| forskolin | 0.349 | −0.570 |
| 1,9-dideoxyforskolin | – | −0.561 |
| 7-deacetylforskolin | 0.346 | −0.574 |
| | Phenyl Glycine-*o*-carboxylic Acid | |
| forskolin | 0.349 | −0.570 |
| 1,9-dideoxyforskolin | – | −0.561 |
| 7-deacetylforskolin | 0.346 | −0.574 |

| | Corresponding Charges on Interacting Centers of the Ligand | |
|---|---|---|
| | charge on sulfonyl/carbonyl oxygen of ligand (interacting with OH at first position) | charge on acidic hydrogen of ligand (interacting with carbonyl group at 11th position) |
| *N*-propinoyl aspartic acid | −0.481 | 0.347 |
| phenyl glycine-*p*-sulfonic acid | −0.252 | 0.330 |
| phenyl glycine-*o*-carboxylic acid | −0.656 | 0.285 |

The IEs of 1:2 complexes of forskolin with ligands clearly indicate better affinity toward forskolin. The gas phase calculations did not give the complete picture of the affinity of forskolin toward the adsorbents, but it surely gives an insight about the possible

interacting centers and most importantly the steric effects, if any, on the interactions. The distances between the interacting centers of forskolin with each ligand's functional groups are within hydrogen bonding distances (Table 2).

Even in solvated conditions, simulated by acetonitrile and methanol molecules, the interaction of forskolin is apparently stronger with the ligands, which is reflected in higher IEs (Table 1) and reduced distances between the interactive centers (Table 2). PGPSA, in fact, showed interaction with 7-deacetylforskolin comparable to that with forskolin. The selectivity of the ligands toward forskolin over 1,9-dideoxyforskolin is clearly evident from these calculations in a vacuum as well as in solvated conditions for the three ligands. The charges on the interactive centers were estimated from the optimized geometries and are given in Table 3. The electrostatic interactions between the interacting centers are mainly responsible for the affinity of forskolin or its analogues toward the ligands. It was also observed that solvents do not affect the charges on the interacting species and were similar to those obtained in a vacuum. The solvent may, however, influence the distance between the interacting centers by forming competitive hydrogen bonds with interacting species. Intermolecular hydrogen bonding among the solvent molecules and intermolecular hydrogen bonding between the solvent and the solute could be responsible for differential solvation of forskolin and its analogues. Owing to two-point attachment and greater electrostatic interaction between the interacting centers, forskolin showed a preferential affinity for the ligands. In methanol solutions, too, the observed interactions of forskolin or its analogues with ligands were similar to those observed in acetonitrile for PGOCA and PGPSA.

The difference in interaction in two solvents lies in the distances between the interactive centers. The MM calculated distances are greater in acetonitrile than in methanol for the forskolin analogues, indicating their poor sorption than forskolin. This difference in distances is reduced in methanol solutions, indicating relatively poorer separation in methanol from each other. Methanol, being an alcoholic solvent, exhibits a greater solvation effect in terms of intermolecular hydrogen bonding with forskolin and its analogues. The distances in interactive centers in methanol solutions, however, are still within H-bond distances.

The molecular simulation results suggest that the intermolecular hydrogen bonding exhibited between the carbonyl/sulfonyl oxygen atoms of the ligand with the hydroxyl group of forskolin is stronger than the hydrogen bonding between two hydroxyl groups. Although such strong interactions are observed for forskolin with all three ligands, among the analogues there was a difference in the order of interaction. 7-Deacetylforskolin showed greater interaction than 1,9-dideoxyforskolin in acetonitrile solutions for all three ligands. The number of points of attachment is relatively less in 1,9-dideoxyforskolin due to the absence of hydroxyl groups, thus leading to its reduced affinity toward the ligand. 7-Deacetylforskolin showed almost the same affinity toward PGPSA as forskolin, suggesting strong interaction of amino acetyl group of the ligand with the hydroxyl groups at the sixth and seventh positions.

The experimental IR spectrum of PGPSA loaded polymer after complexation with forskolin revealed lowering of stretching frequencies of O−H, C−N, and C−H by 9, 2, and 8 $cm^{-1}$, respectively. The IR spectrum of PGOCA loaded polymer also, after complexation with forskolin, showed reduction in the IR stretching frequencies of O−H, C═O, C−N, and C−H by 11, 7, 4, and 11 $cm^{-1}$, respectively. Hydrogen bonding alters the force constant of both these groups; thus, the frequencies of both stretching and bending vibrations are altered.[36]

The heat of formation was calculated using the VAMP module of Material Studio.[37] In the case of the PGPSA ligand, the heat of formation for the forskolin−polymer pair (−7.91 kcal/mol) was higher than those for the 7-deacetylforskolin−polymer (−6.62 kcal/mol) and 1,9-dideoxyforskolin−polymer (−1.44 kcal/mol) pairs. A lower decrease in the heat of formation in the case of the last indicates that it is thermodynamically less stable.

In the case of the PGOCA ligand, the decrease in the heat of formation for the forskolin−polymer pair (−39.49 kcal/mol) was far greater than that for the 1,9-dideoxyforskolin−polymer pair (−8.18 kcal/mol) and significantly lower for the 7-deacetylforskolin−polymer pair (−3.87 kcal/mol). Both PGPSA and PGOCA showed better interaction with forskolin and thus could be useful for selective adsorption of forskolin.

The entire exercise of molecular modeling suggests that all three ligands can be used for selective adsorption of forskolin and also implies use of acetonitrile as a better solvent for purification of forskolin by selective adsorption on the ligand loaded polymer.

## MATERIALS AND METHODS

All the solvents and chemicals were of analytical grade and were procured from SD Fine Chemicals, Mumbai. Dried roots of *C. forskohlii* were obtained from Godavari Biorefineries Ltd., Mumbai. Chloromethylated polystyrene was obtained from Ion Exchange (I) Ltd., Mumbai. The details of the polymeric adsorbent matrix, experimental methods, and analytical techniques were reported earlier.[31]

**Extraction with Organic Solvent of Coleus Roots.** The extraction experiments were conducted in a fully baffled cylindrical glass vessel of 250 $cm^3$ capacity equipped with a four flat blade turbine impeller (2 cm i.d.). The pulverized roots of *C. forskohlii* (0.8−1.0 mm) were suspended in methanol (150 $cm^3$) with 10% (w/w) solid loading. The suspension was agitated vigorously at 1200 rpm at 30 °C for 2 h and then filtered to recover the solution for further processing.

**Decolorization of Crude Forskolin Extracts Using Alumina.** Crystallization of crude forskolin extract and its subsequent decolorization on alumina has been reported in our previous work.[31] All other colored impurities were retained by the alumina which did not elute out with acetonitrile. The eluate obtained after decolorization contained forskolin (78%), 1,9-dideoxyforskolin (6.5%), and 7-deacetylforskolin (15%). The eluate was further used for recovery of forskolin by separation from other two analogues.

**Synthesis of Ligand Selective for Forskolin.** Ligands selective for forskolin were synthesized via acetylation and condensation reactions as shown in Figure 3.

*a. Synthesis of N-Propinoyl Aspartic Acid.* A mixture of disodium salt of aspartic acid and propionic anhydride was stirred in a stirred vessel at 1200 rpm for 6 h at 30 °C. Three times excess of propionic anhydride was taken since the reaction was conducted in water. After 6 h, the pH of the mixture was reduced to 2 by slow addition of 50% sulfuric acid to crystallize *N*-propinoyl aspartic acid (NPAA) from the reaction mixture. The product was filtered and washed with 50 $cm^3$ of methanol to remove traces of propionic anhydride. The product was recovered with 92% purity and 54% yield. The lower purity was due to the presence of sodium sulfate, which is difficult to separate as

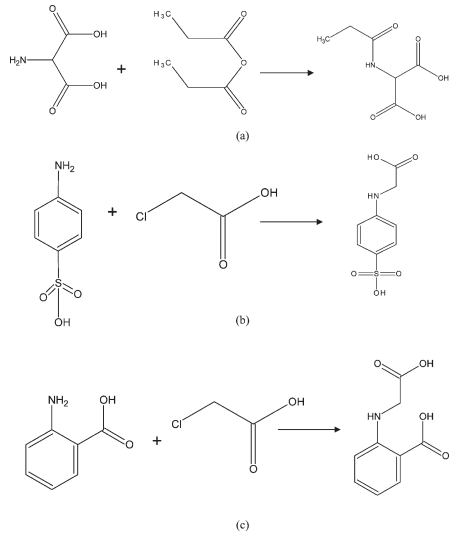

**Figure 3.** Chemical synthesis of various ligands: (a) *N*-propinoyl aspartic acid ligand (NPAA); (b) phenyl glycine-*p*-sulfonic acid (PGPSA); (c) phenyl glycine-*o*-carboxylic acid (PGOCA).

both (NPAA and sodium sulfate) are water soluble. However, it was assumed that sodium sulfate will not have any effect on the loading on the chloromethylated polymer.

*b. Synthesis of Phenyl Glycine-o-carboxylic Acid.* A mixture of anthranilic acid (7.1 g), chloroacetic acid (4.9 g), and sodium carbonate (8.9 g) dissolved in water was stirred for 3 h at 90 °C. The reaction mixture was rendered acidic by addition of concentrated HCl solution to precipitate the product. The solution was allowed to stand overnight at 30 °C for complete precipitation. Phenyl glycine-*o*-carboxylic acid (PGOCA) that crystallized out from the reaction mixture was filtered, washed with 50 $cm^3$ of methanol, and dried in an oven at 60 °C for 1 h with 94% purity and 58.4% yield.[38] PGOCA was obtained in 94% yield after repeated crystallization (twice) using methanol. However, the final product showed the presence of 2.85% unreacted anthranilic acid and 1.14% unreacted chloroacetic acid.

*c. Synthesis of Phenyl Glycine-p-sulfonic Acid.* Sulfanilic acid was condensed with chloroacetic acid to give phenyl glycine-*p*-sulfonic acid (PGPSA). A mixture of sulfanilic acid (5 g), chloroacetic acid (5 g), and sodium carbonate (10 g) was dissolved and stirred in water for 3 h at 90 °C. The reaction mixture was rendered acidic with concentrated HCl to precipitate the product. Unlike PGOCA, no instaneous precipitation was observed; the reaction mixture was cooled to 10 °C for 12 h. The product that crystallized out was filtered, washed with 50 $cm^3$ of methanol, and then dried in an oven at 60 °C for 1 h. Nearly 100% pure PGPSA was recovered but with only 36.6% yield.

**Loading of Ligand on Chloromethylated Polymer.** The ligands were loaded on chloromethylated polystyrene matrix cross-linked with divinylbenzene in dimethyl sulfoxide (DMSO) solutions.[39] The reaction was conducted in a well-stirred fully baffled cylindrical reactor vessel at 90 °C for 24 h. In the case of NPAA, the ligand (2.89 g) was initially dissolved in 20 $cm^3$ of water followed by addition of 50 $cm^3$ of DMSO and then 2 g of polymer beads. In the case of PGOCA and PGPSA, 2.7 g of each ligand was dissolved in 60 $cm^3$ of DMSO followed by addition of 2.5 g of polymer beads. HCl liberated in the reaction was neutralized by triethylamine. After completion of the reaction, the polymer was decanted and subjected to three water washes followed by a 60 mL methanol wash. The polymer was then dried in oven at 40 °C and then subjected to analysis using a Perkin-Elmer FTIR spectrometer (Spectrum BX-II) in a KBr pellet. Energy dispersive X-ray (EDX) analysis for ligand loaded polymer was carried out for loading characteristics based on the amount of chloride group/ions using a JEOL scanning electron microscope.

**Batch Equilibrium Sorption of Forskolin.** Batch adsorption studies were carried out at 30 °C by equilibrating 0.5 g of ligand loaded polymer in stoppered conical flasks with forskolin solutions (20 $cm^3$) of known concentration (100−5000 ppm) in acetonitrile, methanol, and acetone. The adsorbed amount of forskolin was estimated from the residual concentration of forskolin in the solution.

**Column Studies of Ligand Loaded Polymer Selective for Forskolin.** The ligand loaded polymer (3.15 g) was packed in a glass column of 12.5 cm length and 1.3 cm diameter. The top and the bottom zones of the column were packed with glass beads. The feed solution was pumped through the packed adsorption bed by a peristaltic pump at a flow rate of 0.5 $cm^3$/min in upward direction. Samples were collected at regular time intervals at the exit of the column for 1 h and analyzed by HPLC. Once the column was saturated, as indicated by leakage of forskolin in the effluent of the column at the same concentration as the feed, the solution flow was replaced by pure solvent. The adsorbed forskolin on the polymer bed was desorbed with an upward flow of the regenerating solvent at the rate of 0.5 $cm^3$/min. Samples were also collected during the desorption stage and were subjected to HPLC analysis.

## RESULTS AND DISCUSSION

**Characterization of Ligands.** Mass spectral (MS) analysis showed a major $[M - H^+]$ peak with $m/z$ 194 and 231 for PGOCA and PGPSA, respectively. The melting point from a differential scanning calorimeter (DSC) revealed a sharp single peak at 250 °C for PGPSA, whereas for PGOCA a major peak was obtained at 210 °C along with a minor peak for unreacted anthranilic acid at 137 °C. The IR spectrum of NPAA showed N−H stretching band at 3418 $cm^{-1}$ corresponding to a secondary amino group. The shift in N−H stretching from 3019 $cm^{-1}$ in aspartic acid to 3418 $cm^{-1}$ in the ligand indicates conversion of a primary amino group to a secondary amino group. The IR spectrum of PGOCA also revealed N−H stretching at 3372 $cm^{-1}$ corresponding to a secondary amino group, thereby confirming condensation of the primary amino group with chloroacetic acid. In the case of PGPSA, the IR analysis revealed N−H stretching at 3520 $cm^{-1}$ and sulfonyl stretching at 1168 $cm^{-1}$. The presence of carbonyl stretching at 1728 $cm^{-1}$ confirms the condensation of the amino group of sulfanilic acid with chloroacetic acid. Since the MS and DSC analyses showed a single peak for PGPSA, the product was considered to be pure with no impurities.

The ligands loaded on the chloromethylated polymer were also characterized by IR spectroscopy and EDX analysis. In the case of NPAA, based on the residual concentrations of the ligands in the solution, 90% of the reacting groups on the polymer were loaded with the ligand. The IR analysis of NPAA loaded polymer revealed O−H stretching at 3420 $cm,^{-1}$ C−N stretching at 1218 $cm^{-1}$, and a very small peak for C−Cl stretching at 669 $cm,^{-1}$ thereby confirming loading of the ligand on the polymer. Similar IR spectra were obtained for PGOCA and PGPSA with additional carbonyl and sulfonyl stretchings at 1690 and 1168 $cm,^{-1}$

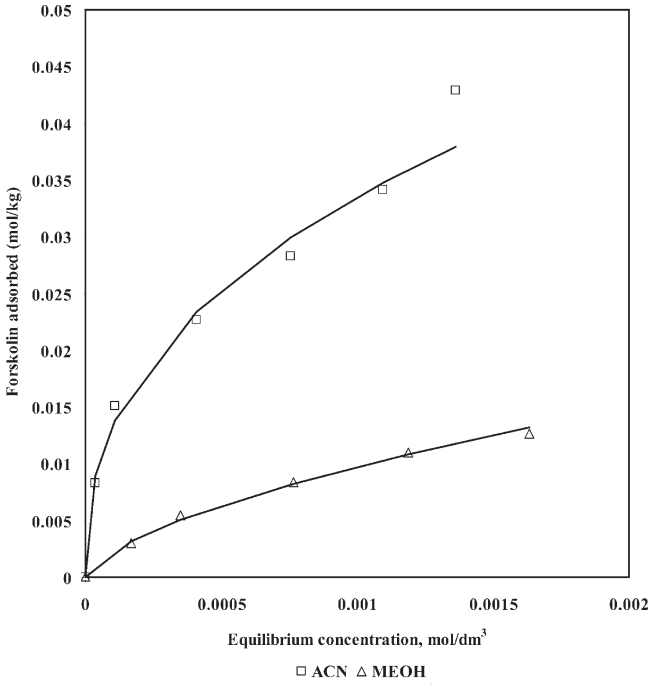


**Figure 4.** Freundlich adsorption isotherm of forskolin for NPAA loaded polymer.

respectively, but no C−Cl stretching at 669 $cm^{-1}$, thereby suggesting complete loading of the ligand on the polymer. However, the EDX analysis revealed 7 and 5% chloride ions on the PGOCA and PGPSA loaded polymers, respectively.

**Batch Equilibrium Sorption Study of Forskolin.** The experimental adsorption data were fitted in the Freundlich adsorption isotherm

$$q = k_F C_e^{1/n} \quad (1)$$

where $k_F$ is a Freundlich constant that is taken as an indicator of adsorption capacity. The extent and degree of adsorption is described by $1/n$; the adsorption is favorable for $1/n$ comprising between 0.1 and 1.[40−42] $C_e$ is the equilibrium concentration in moles per cubic decimeter; $q$ is the moles of forskolin adsorbed per kilogram of adsorbent. The lines in Figures 4−6 are the fitted curves from eq 1. The fitted values of $k_F$ and $1/n$ are reported in Table 4 for acetonitrile, methanol, and acetone solutions for all three ligands. The low values of $k_F$ for NPAA loaded polymer suggest poor adsorption affinity for forskolin. The values of $k_F$ for PGOCA loaded polymer in acetonitrile solutions are much higher compared to those in methanol and acetone solutions, indicating significantly higher adsorption from acetonitrile solutions. The batch adsorption of pure forskolin on all three ligands suggested a higher uptake from the acetonitrile solutions compared to that from methanol and acetone solutions. However, the uptake was found to be very low for the NPPA ligand as shown in Figure 4. The steric hindrance to the interaction between the forskolin and the ligand sites in the polymer cannot be ruled out because of the cross-linking in the polymer structure. In the case of PGPSA and PGOCA loaded polymers, the uptake of pure forskolin was in the order acetonitrile > methanol > acetone, as shown in Figures 5 and 6.

The adsorption of an adsorbate on a given adsorbent has to compete with its solvation in the solution depending upon relative

**Table 4. Freundlich Adsorption Parameters of Forskolin for Different Ligands**

| | $1/n$ | $k_F$ |
|---|---|---|
| | NPAA Loaded Polymer | |
| acetonitrile | 0.39 ± 0.05 | 0.52 ± 0.28 |
| methanol | 0.59 ± 0.02 | 0.74 ± 0.05 |
| | PGPSA Loaded Polymer | |
| acetonitrile | 0.72 ± 0.035 | 13.2 ± 2.96 |
| methanol | 0.55 ± 0.030 | 2.05 ± 0.28 |
| acetone | 0.46 ± 0.007 | 0.91 ± 0.06 |
| | PGOCA Loaded Polymer | |
| acetonitrile | 0.98 ± 0.012 | 78.25 ± 5.12 |
| methanol | 0.79 ± 0.06 | 16.28 ± 2.82 |
| acetone | 0.74 ± 0.025 | 8.02 ± 1.57 |

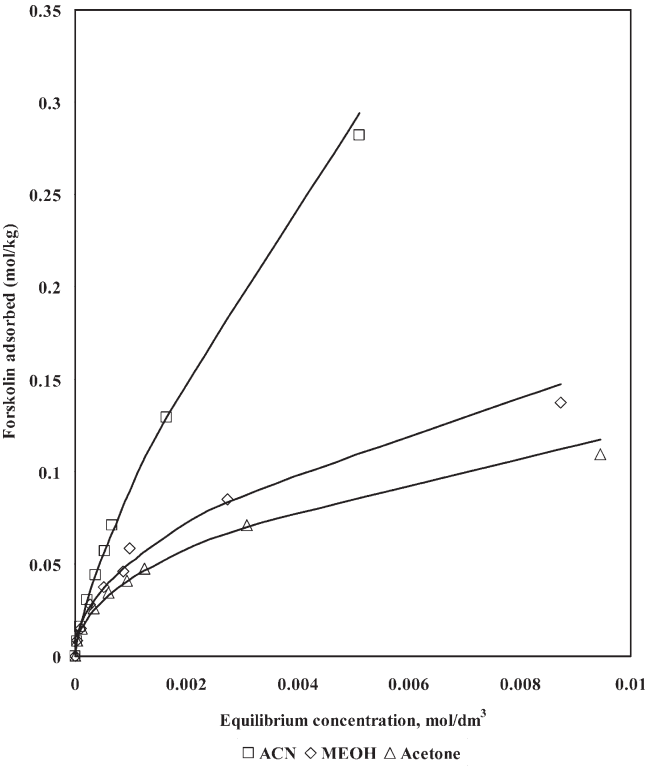


**Figure 5.** Freundlich adsorption isotherm of forskolin for phenyl glycine-*p*-sulfonic acid loaded polymer.

interactions of the adsorbate with the solvent. If a solute is preferably solvated by a solvent, its adsorption tendency becomes weaker while, in poorly solvated conditions, the tendency to be adsorbed by moving out of the solution is stronger. The preferential solvation should also affect the solubility of forskolin in different solvents. The solubility of forskolin in acetonitrile, methanol, and acetone was determined independently to be 0.026, 0.14, and 0.12 mol/$dm^3$, respectively.

Since forskolin is less soluble in acetonitrile compared to methanol and acetone, its adsorption tendency from acetonitrile solutions is higher and thus higher adsorption of forskolin is observed in acetonitrile solutions compared to that from acetone

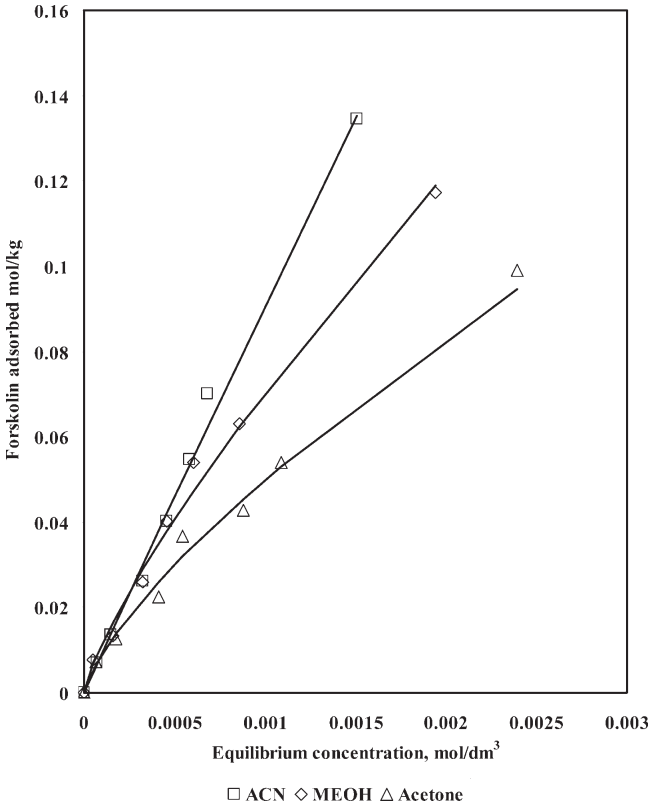


**Figure 6.** Freundlich adsorption isotherm of forskolin for phenyl glycine-*o*-carboxylic acid loaded polymer.

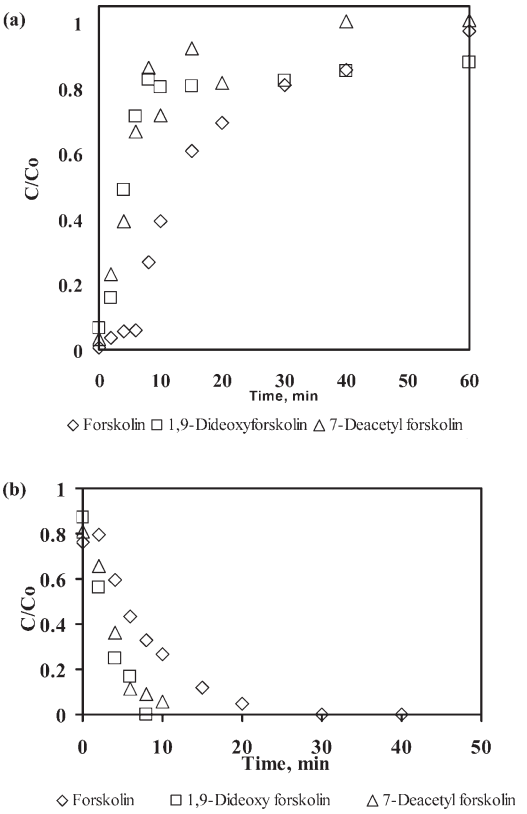


**Figure 7.** Adsorption/desorption of forskolin and its analogues on phenyl glycine-*p*-sulfonic acid loaded polymer in acetonitrile solution.

and methanol solutions. The solubility of forskolin is the highest in methanol, which in turn gives rise to a greater solvation of forskolin in methanolic solutions and thereby leads to lower adsorption on the polymer. In the case of acetone, the solubility of forskolin is slightly lower than that in methanol but the forskolin uptake was also lower than that from methanol. Acetone with its carbonyl group exhibits strong intermolecular hydrogen bonding with hydroxyl group of forskolin which may be stronger than hydrogen bonding interactions with the ligand loaded on the polymer. However, in the case of PGOCA, there was only marginal difference among the three solvents unlike that observed with PGPSA. The two functionalities of the ligand, namely amino acetyl and carboxyl groups, are in close vicinity unlike the functional groups of PGPSA, which may hinder adsorption of forskolin due to the steric effect and thereby have a lesser influence of the solvation in the different solvents.

For all three ligands, the uptake of forskolin was much lower than the known capacity of the ligand loaded polymer. The much lower capacity indicated inaccessibility of ligand sites either inside the polymer or due to reduced surface area and pore size of the polymer after functionalization with the ligands. In our previous study with diethanolamine loaded polymer,[31] the surface area of the polymer reduced from 29.03 to 24.01 $m^2/g$ whereas the pore size drastically reduced from 109 to 67 Å after functionalizing it with diethanolamine. As ligands are bulkier in size, their loading on the polymer significantly can reduce the surface area, pore size, and pore volume which in turn together reduce the adsorption capacity. Since *N*-propinoyl aspartic acid showed very poor uptake of forskolin, the experimental column studies were not carried out using the same.

**Column Studies.** These column experiments were conducted to verify the selectivity of the new ligands toward forskolin because in the previous report the ligand was designed for selectivity toward the forskolin analogues. The eluate obtained after decolorization of the crude forskolin extract through alumina column mainly contained forskolin and its analogues and, therefore, was used as a feed solution for the adsorption on the ligand loaded polymer in a column.

The feed solution contained nearly 78% (w/w) forskolin, 15% (w/w) 7-deacetylforskolin, and 6.5% (w/w) 1,9-dideoxyforskolin, respectively, with only trace amounts of all other impurities. The concentrations of forskolin and its analogues are shown as dimensionless concentration ($C/C_0$) vs time in Figures 7a and 8a for two ligand loaded polymers. The time was counted from the elution of the solution at the exit of the column. In the case of PGPSA loaded polymer, the breakthrough time for the forskolin analogues was 2 min, whereas for forskolin it occurred at 6 min. Since the column was short in length and intraparticle diffusion is the controlling step of the sorption, small leakage of the solutes is seen in the first sample itself when 1 bed volume of solution has passed through the column. The breakthrough fronts of forskolin and its analogues are, however, well separated, suggesting preferential sorption of forskolin from mixtures with its analogues. Most importantly, the experimental observations validate the simulation by molecular modeling with forskolin-selective ligands. In the case of PGOCA also the breakthrough times (corresponding to $C/C_0$ = 0.065) for forskolin and 1,9-dideoxyforskolin and 7-deacetylforskolin (corresponding to $C/C_0$ = 0.07) are 4 and 2 min, beyond which the concentration of respective solutes starts increasing. The bed also saturates differently for different solutes.


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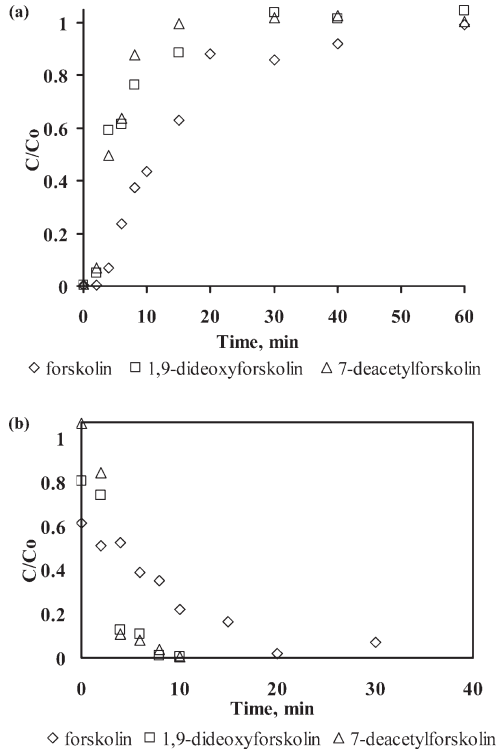


**Figure 8.** Adsorption/desorption of forskolin and its analogues on phenyl glycine-*o*-carboxylic acid loaded polymer in acetonitrile solutions.

For both PGPSA and PGOCA loaded polymers the column bed took more than 40 min for forskolin while for both its analogues it occurred in 15 min (Figures 7a and 8a).

Based on the amounts of forskolin and its analogues in the feed solution initially and in the effluent of the column, the percent uptake of forskolin and its analogues by the polymer was calculated. The adsorption by the polymer from the acetonitrile solution was maximum for forskolin, intermediate for 1,9-dideoxyforskolin, and minimum for 7-deacetylforskolin for the ligand loaded polymer when the column was exhausted. Higher adsorption of forskolin from the feed solution compared to its analogues clearly suggests greater affinity of the polymer toward forskolin.

The sulfur atom of PGPSA loaded polymer is less electropositive (0.05) and imparts higher negative charge on the sulfonyl oxygen (−0.252) which in turn pulls the hydrogen atom attached to the hydroxyl group of forskolin resulting in strong hydrogen bonded complex. In the case of 1,9-dideoxyforskolin, the absence of hydroxyl groups reduces the number of hydrogen bonded interactions, and in the case of 7-deacetylforskolin, probably weak hydrogen bonding interactions are responsible for its lower affinity toward the polymer. During desorption from the loaded column, the forskolin analogues are eluted rapidly compared to forskolin (Figures 7b and 8b). After the analogues were completely desorbed from the polymer bed, a forskolin-rich fraction was obtained with 98% purity.

Forskolin was adsorbed on PGPSA polymer to the maximum extent (89%) followed by 7-deacetylforskolin (15%) and 1,9-dideoxyforskolin (7.85%) from methanol solutions, which shows greater affinity of forskolin even under the strong solvation of methanol. However, forskolin could not be obtained in purer form as both forskolin and its analogues eluted out rapidly almost together by methanol.

PGOCA loaded polymer also adsorbed forskolin from methanol solutions to the maximum extent (89.8%) followed by 7-deacetylforskolin (38.5%) and 1,9-dideoxyforskolin (33.8%), thereby suggesting greater affinity of forskolin toward the ligand. However, forskolin and its analogues were desorbed nearly together, as a result of which forskolin could not be obtained in a more pure form in methanol.

These column studies, however, indicate selectivity of PGPSA and PGOPA toward forskolin. Although PGPSA loaded polymer gave almost 98% pure forskolin during desorption, the yield was only 16% . Still, the process avoids multiple purification steps and at the same time the use of selective affinity adsorbent makes the process simpler and some what more efficient. A longer column can perhaps improve the yield. About 95% pure forskolin was achieved with 46.54% recovery with PGOCA loaded polymer as the desorption of analogues was relatively faster compared to forskolin with acetonitrile. There have been only a few reports providing information about the purity of recovered forskolin. We have reported recovery of 94% pure forskolin by selective adsorption of forskolin analogues on a diethanolamine loaded polymer.[31] The process described by Saleem et al.[21] at the laboratory scale involves five different solvents for adsorption and crystallization of forskolin under reduced pressure with just 0.097% yield. Also, reduced pressures may lead to significant losses of volatile organic solvents. Moreover, no information was provided about the reusability of the adsorbent. If it cannot be regenerated, then the process will also generate a good amount of solid waste. The process of Bhat et al.[19,20] reports no information about the purity and the recovery of the product. In the present system, only the decolorization step uses alumina in a minimum amount to get rid of the colored organic impurities. The use of selective adsorbent has an edge over the chromatographic methods in scale of operation as a larger volume of the solution can be loaded on the column. The use of multiple solvents also needs multiple solvent recovery systems, adding to the cost of the separation.

The synthesis of ligand involves a simple condensation reaction, and intercalating the ligand on the polymer matrix is a straightforward substitution reaction. With a selective and reusable adsorbent and lesser number of solvents, the purification process becomes much simpler compared to the reported adsorbents where the reusability and selectivity toward desired components are major concerns. The proposed approach of developing an affinity adsorbent thorough molecular modeling definitely has an edge over the reported methods for the purification of forskolin in terms of selectivity and simplicity of the purification process.

The PGPSA loaded polymer was tried for repeated runs. The desorption with acetonitrile gave 94, 93, and 80% recovery of 1,9-dideoxyforskolin, 7-deacetylforskolin, and forskolin, respectively, from the column. In the second subsequent adsorption run, the adsorption of forskolin reduced to 48.7% as against 59.6% in the first run but adsorption of 1,9-dideoxyforskolin and 7-deacetylforskolin remained nearly the same. The third adsorption run on the same column gave a further 17% reduction in forskolin using the same solvent which can be improved only by using methanol for desorption. However, the use of methanol reduces the purity of the product as shown above.

## CONCLUSION

The molecular simulation effectively predicts the effect of acetonitrile and methanol on selective adsorption by three poly-

mers loaded with ligands designed on the basis of molecular interactions. The two-point attachment and hydrogen bonding between charged centers of forskolin and its analogues and the ligands such as PGPSA and PGOCA led to recovery of purer forskolin from acetonitrile solutions. The column runs effectively validate the results of molecular modeling. It is possible to involve molecular level understanding of the separation processes in the synthesis of tailor-made specific ligands for better and selective separation of forskolin.

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## NOMENCLATURE

$K_F$ = Freundlich adsorption constant ($dm^{3/n}/mol^{1-1/n}/kg$)
$1/n$ = intensity of adsorption
$C_e$ = equilibrium concentration ($mol/dm^3$)

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