SEPARATION OF UROKINASE FROM CELL CULTURE BROTH

By

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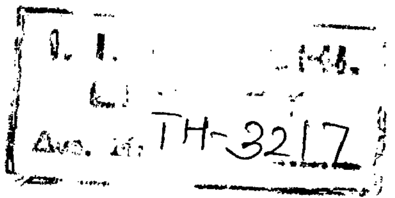

Dedicated

to

My beloved Parents

CERTIFICATE

This is to certify that the thesis entitled "**Separation of urokinase from cell culture broth**", being submitted by **Ms Vibha Bansal** to the Indian Institute of Technology, Delhi, for the award of degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by her under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The research reports and results presented in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

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Sometimes our inner fire goes out but is then blown into flame by another human being. Each of us owes deepest thanks to those who have rekindled this light…Albert Schweitzer.

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ABSTRACT

Urokinase, a valuable thrombolytic drug administered intravenously for the treatment of thromboembolic diseases, is derived majorly from urine for clinical use and research. However, due to extremely low concentrations of urokinase in urine, human cells cultured *in vitro* provide a superior alternative for the production and purification of urokinase. Urokinase production was thus performed using two different anchorage dependent human cell lines, kidney cell line HT1080 and colon cell line HCT116. The broth from HT1080 cell cultures, by virtue of its higher urokinase concentration, was used for the optimization of purification process for urokinase. Para-amino benzamidine is a reversible inhibitor of urokinase and hence finds application for use as affinity ligand in its purification. Benzamidine Sepharose gel was thus prepared in laboratory. Chromatographic separation of urokinase from HT1080 broth using this gel led to a highly pure preparation of urokinase with 181-fold-purification and 60 % recovery of enzyme activity. Other techniques like Sulfo-Propyl Sepharose cation exchange chromatography and immobilized metal affinity chromatography using two different support matrices, Sepharose 4B and polyacrylamide cryogel, were also tested for their efficacy in purification of urokinase. A novel cryogel bioreactor was then designed for the production of urokinase from these cell lines. For this purpose gelatin modified polyacrylamide cryogel monoliths were synthesized and used as macroporous carriers. Cells grew in multilayer on gelatin-cryogel scaffold as observed by scanning electron microscopy. Urokinase activity of the order of 300 PU/ml of culture broth could be achieved in the cryogel bioreactor. On the basis of its larger pore size that facilitated direct loading of cell culture broth on to the column and high-fold purification of

urokinase, Cu(II)-iminodiacetic acid (IDA)-polyacrylamide cryogel was then coupled to the cryogel bioreactor for integrated recovery of urokinase from cryogel bioreactor. The capture of urokinase by Cu(II)-IDA-polyacrylamide cryogel column led to a decrease in urokinase concentration in local environment of the cells thus inducing the cells to produce more urokinase. Initial capture of urokinase from bioreactor using Cu(II)-IDA-polyacrylamide cryogel column and further processing through benzamidine Sepharose column led to a net 3,784-fold purification of urokinase with final yield of 48 %. The urokinase activity in final preparation was 1,17,334 PU/mg of protein. A total of 10^4 Plough units of urokinase (equivalent to 0.7 clinical dose) were harvested from the cryogel bioreactor per day. This preparation was tested for its biological activity by performing a fibrin plate assay. Appearance of a clear zone of lysis indicated that the urokinase so obtained from this integrated production and purification process had retained its physiological role of clot dissolution. Finally another setup was designed to couple benzamidine Sepharose column with hollow fiber bioreactor in which HT1080 cells were actively producing urokinase. Approximately 4.5×10^4 Plough units of urokinase (equivalent to 3 clinical doses) were harvested per day successfully from the integrated set up.

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