STRATEGIES FOR PURIFICATION OF BIOMOLECULES

Om P. Sharma
Biochemistry Laboratory,
Indian Veterinary Research Institute, Regional Station,
Palampur 176 061, H.P. India.
Email: omsharma53@yahoo.com

The primary purpose of purification is obtaining the biomolecule in sufficient amount for studies on determination of chemical/molecular structure and biochemical mechanism of action. This is followed by process development and scale-up for obtaining the biomolecule is bulk amounts for the therapeutic use. Before we embark development of purification protocol we ought to know the bioactivity we are looking for in the bioresource (plant, animal or microbial) and some information about the chemical nature, sample stability window, SSW (temperature, pH, ionic strength, susceptibility to autooxidation), approximate amount in which the bioactive constituent we are looking for is present in the sample. For development of purification of protocol (bioactivity guided purification) different fractions or samples are assayed in order to take a decision which way the protocol should flow for the best separation and rersolution for the best yields. Quite often thin layer chromatography (TLC) is the method of choice for quick monitoring of separation and purification. Decisions about the solvent systems for TLC and chromatographic elution are taken by using Table 1 and 2 as a guide for the polarity of the solvent vis-à-vis the polarity of the constituents of the sample which are to be resolved and separated.

TABLE 1. Eluotropic series of solvents

Solvent	**Dielectric constant**	**Solvent**	**Dielectric constant**
Hexane	1.89	Tetrahydrofuran	7.58
Heptane	1.92	Dichloromethane	9.14
Cyclohexane	2.02	2-Methylpropan-2-ol	10.9
1,4-Dioxan	2.21	Pyridine	12.3
Carbon tertachloride	2.24	Butan-2-ol	15.8
Benzene	2.28	2-Methylpropan-1-ol	17.7
Toluene	2.38	Butan-1-ol	17.8
Acetonitrile	3.88	Propan-2-ol	18.3
Diethyl ether	4.34	Propan-1-ol	20.1
Chloroform	4.87	Acetone	20.7
Formic acid	5.00	Ethanol	24.3
2-Methylbutan-2-ol	5.82	Methanol	33.6
Ethyl acetate	6.02	Water	78.3
Acetic acid, glacial	6.15		

Table 2. Polarity index of some common solvents (in the decreasing order)

Solvent	**Polarity Index**
Water	10.20
Methanol	6.60
Acetic Acid, Glacial	6.20
Acetonitrile	6.20
Acetone	5.40
Pyridine	5.30
Methyl Ethyl Ketone	4.50
Chloroform, Hydrocarbon Stabilized	4.40
Chloroform	4.40
Ethyl Acetate	4.30
2-Propanol	4.30
Tetrahydrofuran, Stabilized	4.20
Tetrahydrofuran	4.20
Methylene Chloride	3.40
Isobutyl Alcohol	3.00
Ether, Anhydrous	2.90
Toluene	2.40
n-Heptane	0.20
2,2,4-Trimehtylpentane	0.10
Hexanes	0.06
Cyclohexane	0.00
Pentane	0.00

The purification simply aims at obtaining the target molecule pure enough in the shortest possible time. Various methods exist to purify proteins and other biomolecules. The characteristics of the the biomolecule of interest such as solubility, charge, size, hydrophobicity, and affinity, along with parameters such as sample type, size, purity, efficacy and abundance of compound in sample will help determine the best chromatography method and media for use.

In many areas, chromatography resins are the media of choice for chromatography applications, but in some cases where resin-based methods have limitations (for example the purification of viruses or large molecules), membranes have proven to be a robust, scaleable and economic alternative. Membranes perform well in certain applications because of their fast flow rates compared to resins. Conventional bead-based chromatography media rely on internal diffusive pores to create large surface areas to achieve high binding capacities. Because of this, the binding capacity of traditional resin columns is flow sensitive due to the diffusive nature of the pores. It is also difficult for large biomolecules such as DNA or viruses to access the internal binding sites of the pores, resulting in low capacity at high flow rates for traditional resins.

Membranes outperform resins when there is a need to purify large molecules or in situations where faster flow is needed because:

a. binding kinetics are not limited by diffusion;
b. binding and elution of large molecules are not restricted by pore size;
c. dynamic capacity is independent of flow rate.

Displacement chromatography and membrane chromatography are the “in-thing” in biomolecule purification. Membrane chromatography is extremely economical because flow rates are significantly faster than traditional resin chromatography, decreasing cycle time and increasing throughput.

Extent of purity required for different applications is given below in Table 3.

Purity	Application
Extremely high > 99%	Therapeutic use, in vivo studies
High 95-99%	Physico-chemical characterization of the molecule, determination of molecular structure
Moderate < 95%	Antigen for antibody production, N-terminal sequencing of proteins

Further Reading

1. http://www.chromatography.amershambiosciences.com/aptrix/upp00919.nsf/Content/LabSep_EduC%5CAboutPurBiom
2. http://www.pall.com/laboratory_38513.asp
3. http://www.acdlabs.com/products/chrom_lab/chromgenius/
4. http://www.doggedresearch.com/chromo/chromatography.htm
5. http://orgchem.colorado.edu/hndbksupport/chrom.html
6. http://www.accessexcellence.org/LC/SS/chromatography_background.html
7. http://ull.chemistry.uakron.edu/chemsep/lc/
8. http://www.shu.ac.uk/schools/sci/chem/tutorials/chrom/chrom1.htm
9. http://matematicas.udea.edu.co/~carlopez/index4.html
10. http://www.separationsnow.com/coi/cda/home.cda?chId=0
11. Protein purification Handbook. www.amershambiosciencs.com. Amersham Biosciences.