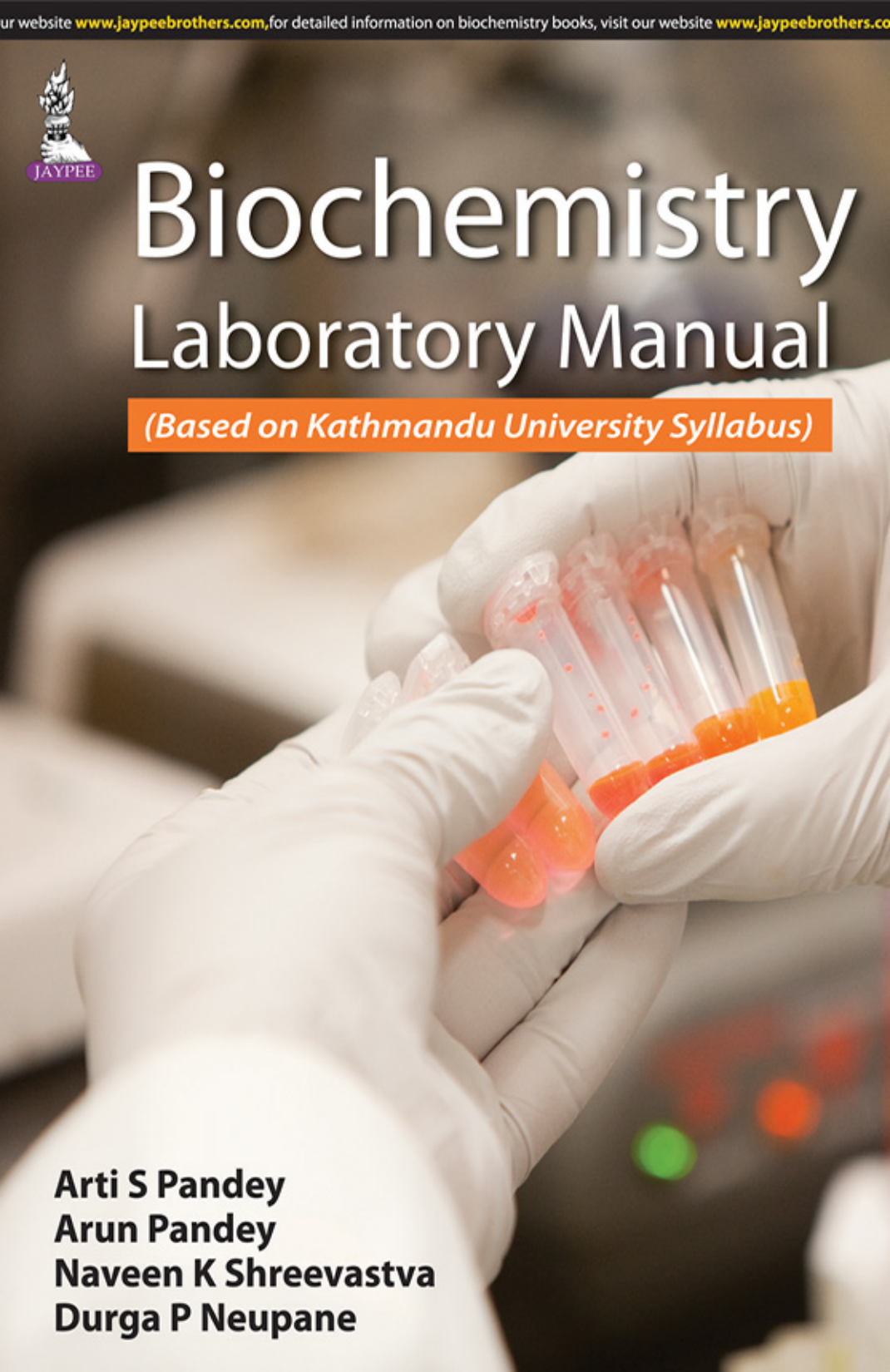

Biochemistry
Laboratory Manual

(Based on Kathmandu University Syllabus)

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Qualitative Tests for Proteins

INTRODUCTION

Normal adults excrete a small amount (upto 160 mg/day) of proteins in urine. This consists of albumin, Tamm-Horsfall protein, some other plasma proteins, glycoproteins, etc. This small amount of protein is not detectable by routine methods. Increased amount of proteins in urine, i.e. proteinuria can be caused by increased glomerular permeability (e.g. albuminuria), reduced tubular reabsorption (e.g. β_2/α_1 microglobulinuria), increased secretion of protein from the renal tract (e.g. Tamm-Horsfall proteinuria) or increased concentration of low molecular weight proteins in the plasma (e.g. Bence Jones proteinuria). Proteinuria may be physiological or pathological. Physiological proteinuria may be seen in last weeks of pregnancy, in severe stress like excessive physical activity, etc. Pathological proteinuria may be seen in conditions such as nephrotic syndrome, pyelonephritis, glomerulonephritis, severe urinary tract infection, etc.

BIURET REACTION (GENERAL TEST FOR PROTEIN)

This test is positive for all compounds containing more than one peptide linkage (tripeptide onwards). All proteins give a positive biuret test.

Principle

Protein reacts with Cu^{2+} ions in alkaline medium to form a blue to violet/purple-colored complex; which is due to formation of co-

ordination complex between Cu^{++} (cupric ions) and peptide bonds of proteins.

Procedure	Observation	Inference
1 ml of protein solution in a test tube +1 ml 5% NaOH + 2 drops of 0.5% copper sulfate solution. Mix well		

HEAT COAGULATION TEST

Principle

Proteins lose their solubility in water as the temperature is raised. This is due to a loss of its tertiary structure or "denaturation". When heated directly, a protein solution will show precipitation or solidifying of protein, a process called coagulation. An example is, heating of egg albumin, which coagulates into a solid, opaque mass.

Procedure	Observation	Inference
Fill 2/3rd of test tube with the protein solution. Heat upper-half of the tube while holding it in a tilted position. Cool and add 2–3 drops of 1% acetic acid		

PRECIPITATION BY SALT SOLUTION (AMMONIUM SULFATE)

The process of protein precipitation by the addition of a neutral salt such as ammonium sulfate is known as salting out. The phenomenon is explained on the basis of dehydration of protein molecules by salts. The cations and anions of salts neutralize the ionic groups on proteins, discouraging interactions with water. This causes increased protein-protein interaction, resulting in molecular aggregation and precipitation. The amount of salt required for protein precipitation depends on the size (molecular

weight) of the protein molecule. In general, the higher the molecular weight of protein, lower is the salt required for precipitation. Larger molecules (e.g. globulins) require half saturation with ammonium sulfate and smaller molecules (e.g. albumin) require full saturation for precipitation.

Procedure	Observation	Inference
A. **Half saturation test**: Take 3 ml protein solution in a test tube and add 3 ml saturated solution of ammonium sulfate. Mix well and keep for 5 min. Filter **Perform the biuret test with the filtrate**: 1 ml of the filtrate + 1 ml of 40% NaOH + 2 drops of 1% $CuSO_4$ solution. Mix well		
B. **Full saturation test with ammonium sulfate and perform biuret test with filtrate**: 3 ml specimen + add solid ammonium sulfate till saturated, filter and perform biuret test **Biuret test**: 1 ml of filtrate + 1 ml of 40% NaOH + 2 drops of $CuSO_4$ solution. Mix it well		

QUESTIONS

1. What is denaturation of protein?

2. **Why does albumin require full saturation with ammonium sulfate for precipitation, whereas globulin requires half saturation?**

3. **Is the presence of albumin in urine normal? Comment.**

4. **When do different types of protein appear in urine?**

5. When are the conditions of hypoalbuminemia and hyperalbuminemia seen? Explain.

Biochemistry
Laboratory Manual

This *Biochemistry Laboratory Manual* is meant for students of the MBBS program that is being carried out in the Medical Colleges under Kathmandu University in Nepal. The manual is based upon the Kathmandu University School of Medical Sciences curriculum for MBBS, and complements what is being taught in theory classes. Clinically relevant questions about the experiments that stress application of a biochemical test in diagnosis are included at the end of each chapter. The manual tries to minimize writing on the part of the students, so that they focus on the results of their experiments, with comments on the results themselves as well as their clinical relevance. The book includes exercises on protein and DNA electrophoresis, which can be taught as a demonstration, or carried out by each student on his own blood samples.

All the authors are faculty members at Kathmandu Medical College (Basic Sciences) in Duwakot, Bhaktapur, Nepal, and teach Medical Biochemistry to MBBS, BDS and Nursing students.

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