

Direct Albumin quantification by NanoDrop and Optical Properties of blood plasma

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Abstract: Human Serum Albumin (HSA) is the most prominent and abundant protein in blood plasma. Deficiency of HSA may cause hypoalbuminemia which is an indicator of cancer. In this study HSA has been quantified in blood samples from nine different cancer patients before (pre) and after (post) first dose of applied chemotherapy drug. Albumin has been quantified by the auto-ranging path length capability of NanoDrop™ 2000. Optical properties of these samples have also been measured. Nonlinear optical properties of human serum albumin from the same human blood plasma has been measured by the Z-scan technique. Change in albumin concentration in the blood serum of a cancer patient undergoing chemotherapy with the corresponding change in nonlinear optical (NLO) response has been calculated. Data analysis reveals a regular change in albumin concentration and a substantial change in the NLO response due to the application of the chemo-drug.

Key Words: NanoDrop™ 2000, HSA, Nuclease Free Water, PBS, 0.9% NaCl, Z-Scan, Plasma, Chemotherapy, Nonlinear Optical Response (NLOR)

Date of Submission: 03-09-2021

Date of Acceptance: 17-08-2021

I. Introduction

The survival of cancer patients depends on their nutritional status. The serum albumin level is a commonly used method for assessing the nutritional status of cancer patients¹. These albumin levels could be affected by multiple factors such as infection, injury etc., resulting in malnutrition such as hypoalbuminemia which is a common scenario of most types of cancer. Although serum albumin is a nutritional factor, hypoalbuminemia acts as an inflammatory marker among colorectal cancer patients². Previous studies have shown that the serum albumin levels in chemotherapy are changed in cancer patients³. Patients with rich nutritional status have high albumin content and so, chemotherapy induced toxicity might not have been observed in them. But patients with hypoalbuminemia show the opposite results⁴.

HSA is a circulating storage and transport medium for many endogenous compounds like several fatty acids, hemin, bilirubin, and tryptophan^{5, 6}. In addition to these HSA also carries exogenous drug molecules⁷, heavy metal ions because of its versatility in binding capability by means of conformational changes⁸⁻⁹. It is the most abundant type of serum protein constituting about 60% of its kind in blood serum that helps vertebrate body to maintain fluid balance¹⁰. HSA exists in monomeric form and is soluble in water. It is synthesized in liver cells called hepatocytes and is transferred to systemic circulation through hepatic plasma where it has a half-life of about 20 days in humans¹¹⁻¹².

Albumin helps to prevent the fluid from leaking out of blood vessels and supplies nutrients to different parts of the body which helps growth and repair of tissues. There is a strong correlation between HSA concentration (Cp) and mortality rate¹³. Standard clinical diagnosis of health conditions include observation of Cp in blood to look for an increase (hyperalbuminemia) or decrease (hypoalbuminemia) of the value, which are associated with hepatic atrophy, heart failure, dehydration, burns, etc.¹⁴. Observations show patients, having serum albumin concentrations lower than 2.5 g/dL, exhibit increased drug sensitivity due to higher amounts of the unbound exogenous drug carried by the albumin¹⁵.

Currently established methods of HSA quantitation involve spectrophotometric and spectrofluorimetric assays¹⁶⁻²⁰. Recent spectrofluorimetric methods utilize the resonance light scattering (RLS) technique where light in the near UV band scattered by the particles gives information of their native state²¹⁻²⁴. NanoDrop2000™ is a micro volume spectrophotometer developed by Thermo Fisher Scientific for quantitation of nucleic acid, purified proteins, etc. with a high degree of accuracy and reproducibility. The Protein A280 method is applied here to determine concentrations of proteins that contain Tryptophan (Trp), Tyrosine (Tyr), Phenylalanine (Phe)