



## Effect of Low Power mm wave Irradiation on sedimentation, coagulation and light absorbance of purified Bovine Serum Albumin

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### Abstract:

Globular protein Bovine Serum Albumin is water soluble and is sensitive to Low Power mm Wave (Micro wave) irradiation leading to Coagulation at the isoelectric Point. The objective of the work was to study the effect of these radiations on the coagulation and light absorbance of purified bovine serum albumin protein. When a heat stress is induced by a Low Power mm Wave, an aggregation of the molecules leading to coagulation occurs; comparatively at high temperature coefficient. The Bovine Serum Albumin Solution maintained at a pH of 3.5 after irradiation by Low Power mm Wave remained clear, the sedimentation constant was multiples almost 8 times (indicating aggregation and agglutination) when compared to the native sample. When the Bovine Serum Albumin sample was irradiated with a frequency of 72.13 GHz, a small change in sedimentation coefficient took place indicating primary coagulation.

When the frequency was increased to 98.52 GHz, there was a rapid increase in the sedimentation coefficient and high level of coagulation indicating almost complete denaturation of Bovine Serum Albumin. As a matter of fact frequency at 114.35 GHz, did not make much change in the sedimentation coefficient and coagulation remained almost constant. Similarly Bovine Serum Albumin treated at 56 °C for 25 minutes showed equivalent coagulation as compared to Low Power mm wave irradiation within the range of 72.13 GHz, to 114.35 GHz, as analysed by U.V/Vis. Spectroscopy (samples treated with Coomassie Brilliant Blue G250) at 595 nm showed relatively similar absorbance.

### Introduction:

Bovine Serum Albumin (also known as **BSA** or **Fraction V**) is a serum albumin Protein derived from ruminants. BSA has 585 Amino acid residues of Molecular weight 66.5 kDa (Fig. 2). It has an isoelectric point of 4.7 at 25 °C in water. Extinction coefficient of 43,824 M<sup>-1</sup>cm<sup>-1</sup> at 279 nm[1].

Dimensions: 140 X 40 X 40 Å<sup>3</sup> (prolate ellipsoid where a = b < c)[2]. BSA has numerous biochemical applications including ELISAs (Enzyme-Linked Immunosorbent Assay), immunoblots, and immunohistochemistry. It is also used as a nutrient in cell and microbial culture. In restriction digests, BSA is used to stabilize some enzymes during digestion of DNA and to prevent adhesion of the enzyme to reaction tubes, pipette tips, and other vessels [3].

In this research paper the effect of microwaves (millimetre wave) on the coagulation of BSA is observed. Microwaves are radio waves which falls under the category of electromagnetic waves with wavelengths ranging from as long as one meter to as short as one millimeter, with frequencies between 300 MHz (0.3 GHz) and 300 GHz.[4] This broad definition includes both UHF and EHF (millimeter waves), and various sources use different boundaries.[5] In all cases, microwaves include the entire SHF band (3 to 30 GHz, or 10 to 1 cm) at minimum, with RF engineering often putting the lower boundary at 1 GHz (30 cm), and the upper around 100 GHz (3 mm).

Microwave radiations with shorter wavelengths are called "Millimeter waves", Terahertz radiation or even T-rays. Definitions differ for millimeter wave band, which the IEEE defines in the frequency range of 110 GHz to 300 GHz.

The microwave frequency band specified by IEEE is as follows:-

Band	Frequency Range (GHz)
L	1 to 2
S	2 to 4
C	4 to 8
X	8 to 10
Ku	12 to 18
K	18 to 26.5
Ka	26.5 to 40
Q	30 to 50
U	40 to 60
V	50 to 75
E	60 to 90 (millimeter waves)
W	75 to 110
F	90 to 140
D	110 to 170

Fig-1 Microwave Frequency Bands

This current research paper is based on observing the heating effects of millimetre wave radiation in a frequency range lying primarily in the E band (60 to 90 GHz) and under extreme conditions extending it up to W band (75 to 110 GHz) on Bovine Serum Albumin (BSA).

Microwave signals are generated by high power sources which use specialized vacuum tubes. These devices operate on different principles from low-frequency vacuum tubes, using the ballistic motion of electrons in a vacuum under the influence of controlling electric or magnetic fields, and include the magnetron (used in microwave ovens), klystron, traveling-wave tube (TWT), and gyrotron. These devices work in the density modulated mode known as velocity modulation, rather than the current modulated mode. This means that they work on the basis of clumps of electrons flying ballistically through them, rather than using a continuous stream of electrons.

Low-power microwave sources use solid-state devices such as the field-effect transistor (at least at lower frequencies), tunnel diodes, Gunn diodes, and IMPATT diodes [6]. The sun also emits microwave radiation, although most of it is blocked by Earth's atmosphere. [7]

#### **Materials and Methods:**

Microwave sources are essential instruments for developing almost all millimeter wave systems and for extending the range of microwave systems. The E8257DSxx series (available from OML, Inc. as the SxxMS-AG series) of external, high power, frequency banded mm-wave source modules, when paired with the high performance Portable Signal Generator (PSG), provide synthesized frequency performance, mm-wave test signals for wavelength bands from 50 to 500 GHz.

These modules have RF input power protection (up to 5W CW) allowing all PSG's with Option 1EU or 1EA (high output power) to provide direct RF drive without the need for external RF power amplifiers. In addition, these modules have been designed to derive DC power directly from the PSG (through the source-module-interface connector), eliminating the need for an external DC power supply. Because these mm-source modules employ frequency multiplication to generate mm-wave frequencies, the output frequency specifications are directly proportional to the characteristics of the PSG driving the module. The E8257D's frequency resolution is typically in the

milli-Hz range that allows a mm-wave carrier frequency of 500 GHz to have a frequency resolution of < 1 Hz.

#### **PSG specifications**

- Wide frequency coverage: 50 GHz to 500 GHz.
- High output power (typically +8 dBm at 110 GHz).
- Direct RF drive from E8257C/D PSG's with Option 1EU or 1EA.
- DC power supplied from E8257C/D via source module interface connector.
- Input power protection.
- Direct frequency control and display on PSG
- FM, PM, and pulse modulation available through mm source module.
- Low phase noise and excellent frequency resolution (< 1 Hz at 500 GHz).

UV/Vis. Spectrophotometer(Sigma Aldrich Spectro 1000) was used with Cuvettes (Sigma Aldrich-Brand UV cuvettes micro centre height 8.5 mm, chamber volume 70-1000  $\mu$ L, window W x h 2.0 mm x 3.5 mm). Micropipette (Sigma Aldrich Volac Volumetric pipette 1 mL vol accuracy 0.008 mm), tips (Sigma Aldrich Eppendorf combitips plus Biopur capacity 1.0 mL). Eppendorfs(Sigma Aldrich-Eppendorf minitube vol. 1.5 mL). BSA(Life Technologies- BSA 150 mg) and Coomassie Brilliant Blue G250 dye( Sigma Aldrich- Brilliant Blue G>90% TLC). Centrifuge (Sigma Aldrich-Eppendorf Microcentrifuge 5424 with 24 place rotor).

#### **Preparation of Bovine Serum Albumin(1mg/ml) [8]:**

1. Reagent: The assay reagent was made by dissolving 100 mg of Coomassie Blue G250 in 50 ml of 95% ethanol. The solution was then mixed with 100 ml of 85% phosphoric acid and made up to 1 L with distilled water. The reagent was filtered through Whatman no. 1 filter paper and then stored in an amber bottle at room temperature. It was stable for several weeks. However, during this time dye may had precipitated from solution and so the stored reagent had been filtered before use.

2. Protein standard: Bovine  $\gamma$ -globulin at a concentration of 1 mg/ml (100  $\mu$ g/ml for the micro assay) in distilled water was used as a stock solution. This was stored frozen at  $-20^{\circ}$ C. Since the moisture content of solid protein may vary during storage, the precise concentration of protein in the standard

solution should be determined from its absorbance at 280 nm. The absorbance of a 1 mg/mL solution of  $\gamma$ -globulin, in a 1-cm light path, is 1.35. The corresponding values for two alternative protein standards, bovine serum albumin and ovalbumin, are 0.66 and 0.75, respectively.

3. Plastic and glassware used in the assay should be absolutely clean and detergent free. Quartz (silica) spectrophotometer cuvettes should not be used, as the dye binds to this material. Traces of dye bound to glassware or plastic can be removed by rinsing with methanol or detergent solution.

4. Poured 500  $\mu$ l of BSA stock solution each in 1.5 ml eppendorf. Labelled them A1, B1, C1 and A2, B2, C2. Poured 500  $\mu$ l of 0.15 M NaCl in each labelled eppendorfs. Filled the remaining 0.5 ml with the assay reagent Coomassie Blue G250.

#### ***Irradiation of millimetre wave on samples A1, B1 and C1:***

All the Samples A1, A2, B1, B2, C1 and C2 were placed in pairs(set A followed by B and then C) inside the cavity resonator after a predetermined period of time and millimetre wave ( RF signal generated from signal generator i.e Agilent PSG generator) was irradiated on each of the above samples.[9]

The effects of millimetre wave irradiation on sample set A were observed at a frequency of about 72 GHz which acted as the resonant frequency of the resonator during irradiation on sample set A.

Similarly, the effects of irradiation on sample set B and C were observed at frequencies of around 98 GHz and 114 GHz.

An important point to note is that the cavity resonator was tuned to three different resonant frequencies i.e 72, 98 and 114 GHz respectively selected from the incoming signal from RF source depending upon the distance required by the millimetre wave to travel to the conducting end and come back to the different samples set A, B and C respectively as a function of velocity of light.

#### ***Preparing Blank to calibrate Spectrophotometer:***

A Blank solution Sample was prepared by mixing 0.15 M NaCl and 0.5ml assay dye. The sample was loaded in two cuvettes and then the baseline was set with absorbance at 595 nm. The same Blank is hence used as a reference for the measurement of absorbance of the protein samples.

#### ***Measuring Absorbance of Sample A1, B1 and C1 at 595 nm:***

Sample A1 was poured in the cuvette and absorbance was noted at 595 nm. Similarly Sample B1 and C1 were poured and absorbance was noted at 595 nm.

#### ***Determination of the Sedimentation Coefficient and Coagulation of the Samples:***

Sample A2, B2 and C2 was centrifuged at 12000g for 10 minutes together and their Sedimentation coefficients were calculated. Coagulation was observed and was related to the discrete Sedimentation Coefficient values hence.

#### **Results and Discussion:**

In a resonator if the distance between the sides is  $d$ , the length of a round trip is  $2d$ . In order to cause resonance, the phase of a sinusoidal wave after a round trip has to be equal to the initial phase so the waves will reinforce. So the condition for resonance in a resonator is that the round trip distance,  $2d$ , be equal to an integral number of wavelengths  $\lambda$  of the wave:

$$2d = N\lambda \quad N \in \{1, 2, 3, \dots\}$$

If the velocity of a wave is  $c$ , the frequency is  $f = c/\lambda$  so the resonant frequencies are:

$$f = \frac{c}{2d} N \quad N \in \{1, 2, 3, \dots\}$$

Sample set A (A1, A2) when irradiated with 72.13 GHz mm wave frequency, showed an almost clear solution by naked eye visualization. When absorbance was measured at 595 nm, it was found to be 0.873(Fig. 3). Sample A2 after centrifugation at 12000g for 10 minutes showed a sedimentation coefficient of 4 S. In case of Sample set B (B1, B2) irradiated with 98.52 GHz showed a drastic agglutination followed by precipitation on cooling; absorbance measured at 595 nm, was found to be 0.187(Fig. 3). Therefore it is inferred that there is a decrease in the absorbance and therefore absorbance is inversely proportional to the agglutinability of the protein. Sample B2 after centrifugation at 12000g for 10 minutes showed a drastic increase in its sedimentation coefficient which was calculated to be 32 S. Sample set C (C1, C2) irradiated with 114.35 GHz, showed agglutination similar to sample set B without any noticeable difference by naked eye visualization; absorbance was measured at 595 nm, was found to be 0.170 (Fig. 1). Therefore it is inferred that although there is a change in the

frequency of the mm wave irradiation, still there was no as such noticeable change in the agglutinability and hence the absorbance change from that of sample B was almost near. Also, it is inferred that the optimum frequency required for maximum agglutinability and hence denaturation would be 98.52 GHz. Sample C2 after centrifugation at 12000g for 10 minutes showed a negligible increase in its sedimentation coefficient from sample B2 which was calculated to be 34 S.

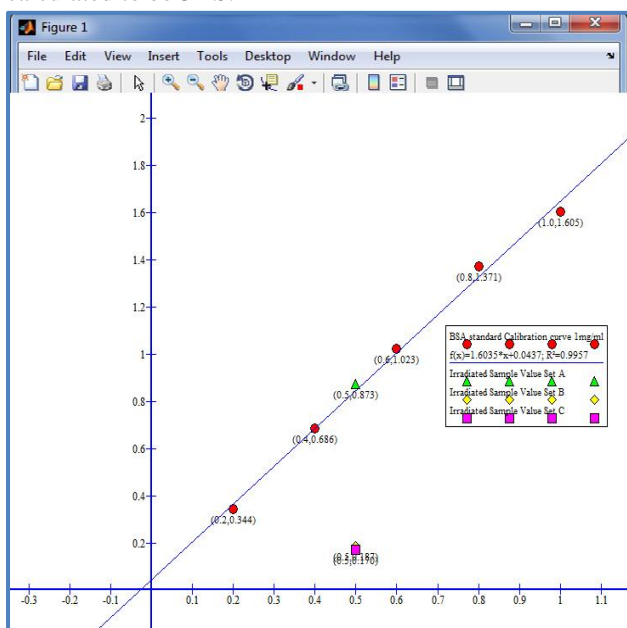


Fig 1: Absorbance plotted against concentration of BSA using MATLAB r2007b

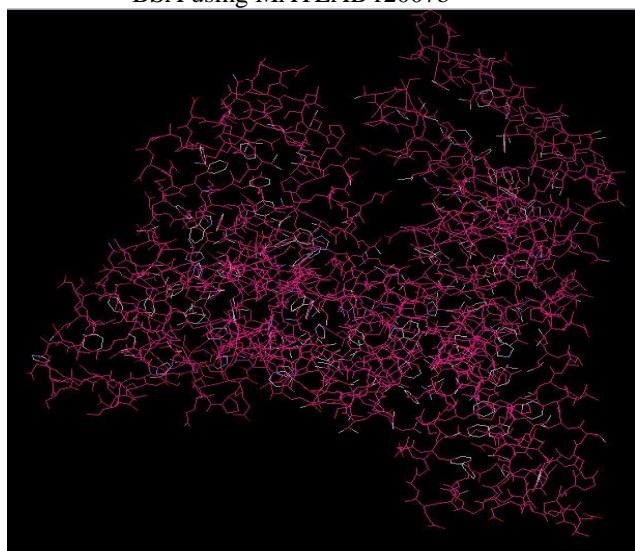


Fig 2: BSA wire frame model visualised in RasMol 2.6

### Conclusion:

This work uses millimetre waves in E band and W band of microwave frequencies specified by IEEE standards lying between 72 to 115 GHz. The basic principle of resonance has been implied in the carried work for generation of desired frequency which has been selected by the cavity resonator. The microwave source that has been used for generation of RF signal is basically a signal generator that generates an amplified continuous RF wave signal which has been applied as input to the hollow end of the resonator. Irradiated samples showed significant agglutinability and a very high sedimentation coefficient. The protein was found to be denatured. Similarly Bovine Serum Albumin treated at 56 °C for 25 minutes showed equivalent coagulation as compared to Low Power mm wave irradiation within the range of 72.13 GHz. to 114.35 GHz.

### References:

- [1] Peters T (1975). Putman FW. ed. The Plasma Proteins. Academic Press. pp. 133–181.
- [2] Wright AK, Thompson MR (February 1975). "Hydrodynamic structure of bovine serum albumin determined by transient electric birefringence". *Biophys. J.* 15 (2 Pt 1): 137–41.
- [3] "BSA FAQ". Invitrogen. Retrieved 19 January 2012.
- [4] Pozar, David M. (1993). *Microwave Engineering* Addison-Wesley Publishing Company. ISBN 0-201-50418-9.
- [5] Goldsmith, JR (December 1997). "Epidemiologic Evidence relevant to RADAR (microwave) effects". *Environmental Health Perspectives* 105(Suppl. 6): 1579-1587
- [6]. *Microwave Oscillator notes* by Herley General Microwave
- [7]. Liou, Kuo-Nan (2002). *An introduction to atmospheric radiation*. Academic Press. p. 2. ISBN 0124514510. Retrieved 12 July 2010.
- [8] Walker John M., 2<sup>nd</sup> Edition, *The Protein Protocols Handbook*. Humana Press ISBN 0-89603-940-4. P.15-20
- [9] Agilent PSG Signal Generators, Brochure, Literature Number 5989-1324 EN.

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