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Research Article

EFFECT OF AMPICILLIN AND CHLORAMPHENICOL ON CHICK SERUM

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ABSTRACT

Objective: Cystatin protein present in chick serum exhibits antimicrobial activity. The present study focuses on the effect of ampicillin and chloramphenicol antibiotics on chick serum and thus verifies Beer–Lambert's law.

Methods: The serum is separated from the cellular matter with the help of a micropipette to get a clean serum sample. The quantification of protein was done by Lowry's method. The antibiotics ampicillin and chloramphenicol stock solution were prepared by 10 mg of antibiotic powder in 10 ml of sterilized water. The statistical analysis of the values obtained was done by SPSS logistics software.

Results: The different values of concentration of serum with absorption showed a linear relationship which verifies Beer–Lambert's law. With an increase in the concentration of protein in chick serum, the absorption also increases, which gives a range of concentration of protein at which ampicillin and chloramphenicol act.

Conclusion: The rise and fall in the absorbance rate of proteins after addition of different antibiotics represent the increase and decrease in the concentration of proteins, respectively. This shows that every antibiotic acts at a particular concentration on the protein of the serum. Therefore, proper doses of antibiotics are recommended by the doctors.

Keywords: Spectrophotometry, Protein, Quantification, Bovine serum albumin, Serum.

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INTRODUCTION

Bovine serum albumin (BSA) is a transport protein found in serum which plays a major role in regulating the circulatory system in the human body [1]. It is a globular protein with 583 amino acids [2]. Its availability, feasibility, and ligand-binding properties give it uniqueness and it is easy to study the properties of the protein [3]. BSA is the most exuberant protein present in plasma [4]. It exhibits many similar features with human serum albumin [5].

Protein nanoparticles of BSA have several biomedical applications in drug delivery system and modifications [6]. The binding capacity of a drug to serum albumin present in human blood has momentous effect on metabolism of drugs throughout the human body [7]. Several commercially prepared protein assay reagents are already available, and for our work, we have used the Lowry's reagent [8]. In the presence of protein, the reagent turns into blue – the concentration of protein present in the sample is directly proportional to the color produced. The blue-colored product absorbs light best at 750 nm, and at this specific wavelength, we have done various measurements [9]. The protein quantification is determined by comparing the optical density values of the sample to the absorption coefficient obtained by plotting a standard curve. Lowry's reagent reacts equally with each type of protein, and hence it does not matter which protein is used for the process of quantification [10]. The silver nanoparticles synthesized in BSA matrix show antimicrobial [11] and antibacterial properties [12]. Chloramphenicol is effective against Gram-positive and Gram-negative bacteria [13] (Fig. 1).

Beer–Lambert's law states that there is a constant relationship between absorbance values and concentration of the sample; as the amount of concentration of protein increases, the absorbance value also increases. Thus, Beer–Lambert's law can only be applied when there is a linear relationship. Beer–Lambert's law is written as: A = epsilon {lc}, where A is the measure of absorbance, l is the path length, c is the concentration, and Epsilon is the molar extinction coefficient or molar absorption or absorption coefficient. The law considers concentration of absorber as a constant value [14]. Cystatin protein in serum of chick has antimicrobial activity. Gram-negative strains such as *Escherichia coli* and *Oligela* sp. were more sensitive to lower concentration of cystatin than Gram-positive bacteria such as *Staphylococcus aureus* [15]. *E. coli* and *Pseudomonas aeruginosa* are substantially inhibited by cystatin even at low concentration [16].

METHODS

Preparation of chick blood sample

Chick blood sample was taken in a sterile tube and centrifuged at 4°C at 4000 rpm, for 10 minutes. Two distinct layers of serum and cellular matter were obtained and 5.5 ml of Lowry's solution was added to each test tube. To mix the solutions properly, it was capped and vortexed briefly. The solution was incubated for 20 minutes at room temperature in dense light. At the last 5 minutes, the diluted form of Folin's reagent was prepared. After the process of incubation, prepared samples were taken and 0.5 ml of diluted Folin's reagent was added to each test tube. The samples of BSA were transferred to a semi-micro disposable cuvette. Spectrophotometer was set in the quantitative mode and at 750 nm. Then, the absorbance values for the standards at the standard mode and the samples at the unknown mode were recorded [17].

Separation of serum from chick blood

The serum was separated from the cellular matter with the help of micropipette to get a clean serum sample. Five test tubes were taken from the test tube stand and the three different concentrations of sample were added to the test tubes.

Preparation of chemicals

The chemicals used in our experiment were Folin's reagent, sodium tartrate, copper sulfate, and two antibiotics – ampicillin and chloramphenicol.

Preparation of solutions for characterization of protein

Using the individual solutions for the Lowry's solution, we prepared the solution and in particular mixed on the day of measurement and readings were taken from a spectrophotometer. $CuSO_4.5H_2O$ and $Na_2C_4H_4O_6.2H_2O$ were dissolved separately in distilled water [18].

Solution A was prepared which is a dilute alkali solution [19].

- Solution A composition (alkaline solution of 500 ml): 2.8598 g NaOH, 14.3084 g Na_2CO_3
- Solution B composition (for 100 ml): 1.4232 g $CUSO_4.5H_2O$
- Solution C composition (for 100 ml): 2.85299 g $Na_2C_4H_4O_6.2H_2O$

Preparation of Lowry's solution

Solution A, solution B, and solution C were mixed with a volume:volume ratio of 100:1:1. The solutions were capped and vortexed properly for homogenization.

Preparation of Folin's reagent (0.1 ml/sample)

A volume of 5 ml of 2N Folin and Ciocalteu's phenol reagent was added to 6 ml of distilled water and mixed properly. The resulting solution is sensitive to light. Hence, we prepared it at the last 5 minutes of the first sample incubation and thus stored it in an amber container. The dilution ratio for the Folin and Ciocalteu's phenol reagent was kept at 1:1 that resulted in a 1N Folin's reagent.

Preparation of antibiotics

Ampicillin and chloramphenicol

A composition of 10 mg of antibiotic powder was provided and made it up to 10 ml by sterilized water. This was used as a stock solution to prepare three different concentrations of antibiotics as: 100 µl, 200 µl, and 1000 µl.

Statistical analysis

All the experiments of concentration of protein and absorbance values were conducted. Different concentrations of two antibiotics, ampicillin and chloramphenicol, were used [20]. The mean and degree of freedom were considered for each factor, and one-way ANOVA was used to obtain the results. p=0.05 was set as level of significance, and the results were obtained as significant in case of p<0.05 [21].

SPSS logistics

The frequency table and corresponding bar charts of the comparison table of composition of chick serum to that of different vertebrates were obtained by SPSS logistics software. The accuracy and frequency table of comparison of color produced by serum of different vertebrates were also obtained in a similar manner [22]. The corresponding pie chart of frequencies was plotted in a similar way using IBM SPSS STATISTICS 24 software [23].

Calculations

Calculations from BSA graph (Graph 1)

From the Beer–Lambert's law, the value of epsilon was calculated. This was done by taking the absorbance and coefficient of BSA sample.

A= Epsilon *{L*C} where Epsilon is absorption coefficient
Slope from the BSA graph: 0.0089/mg/Lcm
Epsilon=slope/L
=0.0089/mg/Lcm.

Taking this Epsilon value and using the Beer–Lambert's law, the protein concentration of the sample with and without antibiotics was prepared.

RESULTS AND DISCUSSION

The comparison between the colors of serum of different vertebrates clearly indicates that the color of chick serum is yellow. This is mainly due to the presence of yellow lipochrome (serum lutein) extracted by ethyl alcohol [24] (Table 1).

A comparison between composition of serum and serum proteins of different vertebrates conspicuously indicated that, at identical pH, ionic strength, and dilution, the ionic mobility of chick serum is highest in comparison to other vertebrates. The reason behind high mobility is the presence of high-mobility group proteins isolated from estrogen-stimulated chick oviduct [25] (Table 2).

As the concentration of protein increases, the absorption of light by protein also increases linearly. This is in accordance with Beer–Lambert's law and hence its verification; as the concentration of protein increases, the absorption also increases since absorption is directly proportional to concentration according to Beer–Lambert's law (Table 3).

The sample without the addition of antibiotics showed a linear relationship with the concentration of serum (Table 4).

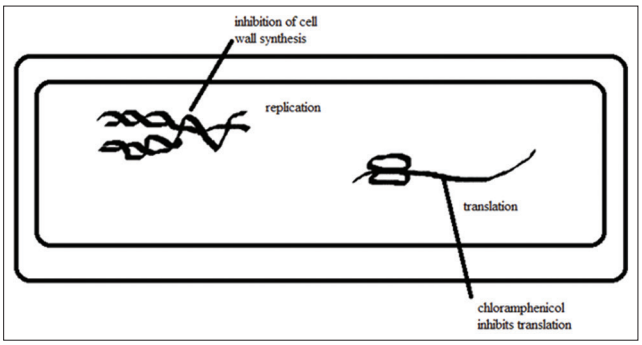
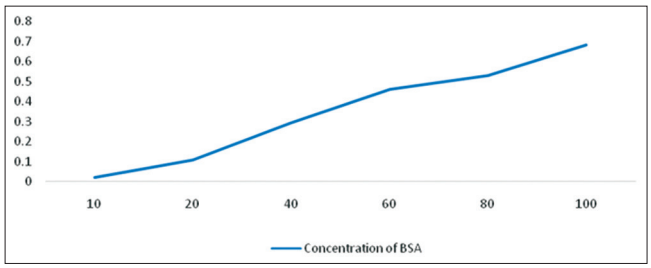


Fig. 1: Action of antibiotics on bacteria



Graph 1: Obtained from values of Table 3: Graph of concentration of bovine serum albumin protein with absorbance values

Table 1: A comparison table between colors produced of serum sample of chick and other vertebrates

Serum sample	Color produced
Chick	Orange red
Pigeon	Orange red
Dove	Orange red
Tortoise	Orange red
Toad	Very faint yellow
Lizard	Very faint yellow
Other vertebrates	Colorless

Table 2: Differences in composition of serum in different animals

Sample taken	pH	Ionic strength	Dilution	Mobility
Human	7.3	0.15	1:3	4.7
Monkey	7.3	0.15	1:3	4
Dog	7.3	0.15	1:3	3.8
Cat	7.3	0.15	1:3	5.3
Chick	7.3	0.15	1:3	6.2
Pigeon	7.3	0.15	1:3	4.4

The major discernment for this effect is that, when the concentration increases, the number of molecules to interact with light also increases, hence increasing the absorption rate with an increase in concentration value (Table 5).

Chloramphenicol antibiotic is bacteriostatic in nature. It inhibits the process of mitochondrial protein synthesis [26]. Addition of this antibiotic on chick serum indicates the role of chloramphenicol on chick serum and also validates Beer–Lambert's law (Table 6).

Ampicillin is a beta-lactam antibiotic which penetrates inside the cell membrane of a bacterial cell. It thus inhibits the growth of bacteria causing cell lysis [27]. Thus, increasing the concentration of antibiotics on chick serum helps us to study the increase or decrease in the concentration of protein present in serum. With increase in the concentration of protein in chick serum, the absorption also increases which gives a range of concentration of protein at which ampicillin acts (Table 7).

The l-erythro isomer of chloramphenicol inhibits the growth of bacteria by blocking protein synthesis [28]. By increasing the concentration of antibiotic, we can interpret the range of serum protein at which antibiotic chloramphenicol acts (Table 8).

Table 3: The values obtained from spectrophotometer by increasing the concentration of protein

Concentration of protein (mg/l)	Absorption
10	0.021
20	0.109
40	0.293
60	0.463
80	0.532
100	0.685

Table 4: The different values obtained with increasing concentration of chick serum without the addition of antibiotics with absorption

Absorption at different concentrations of serum	
Concentration of serum (mg/l)	**Absorption**
10	0.991
20	1.041
40	1.259
60	1.362
80	1.651
100	1.594

Table 5: A comparison between the three different concentrations of antibiotic ampicillin and the absorption values

Absorption at different concentrations of serum	
Concentration of serum (mg/l)	**Absorption**
Concentration of antibiotics: 100 µg/ml	
10	0.769
20	1.340
40	1.480
Concentration of antibiotics: 200 µg/ml	
10	0.683
20	1.608
40	1.070
Concentration of antibiotics: 1 mg/ml	
10	1.467
20	1.335
40	1.423

The one-way ANOVA results in the addition of ampicillin antibiotic at different concentration showed $p<0.05$. Thus, we reject the null hypothesis stating that the sum of means is significant with respect to the corresponding p value. The variation between sample means/ variation within the samples signifying the F-ratio depicts that the variation between the given values of concentration of antibiotic and absorption value is significant [29] (Table 9).

One-way ANOVA test on the values obtained by the concentration of chloramphenicol antibiotic on chick serum showed $p<0.05$. Thus, we reject the null hypothesis and conclude that the sum of the means is

Table 6: A comparison of three different concentrations of chloramphenicol antibiotic addition on chick serum

For chloramphenicol	
Absorption at different concentrations of serum	
Concentration of serum (mg/l)	**Absorption**
Concentration of antibiotics: 100 µg/ml	
10	1.077
20	1.358
40	1.630
Concentration of antibiotics: 200 µg/ml	
10	0.895
20	1.560
40	1.380
Concentration of antibiotics: 1 mg/ml	
10	0.817
20	1.380
40	1.496

Table 7: A comparison between different concentrations of antibiotic ampicillin and the absorption values

With antibiotics: For ampicillin	
Concentration of protein (mg/l)	**Absorption**
Concentration of antibiotics: 100 µg/ml	
86.40	0.769
150.56	1.340
166.2	1.480
Concentration of antibiotics: 200 µg/ml	
76.74	0.683
180.67	1.608
120.22	1.070
Concentration of antibiotics: 1 mg/ml	
164.83	1.467
150	1.335
159.88	1.423

Table 8: Different absorption values of serum protein on addition of chloramphenicol

For chloramphenicol	
Concentration of protein (mg/l)	**Absorption**
Concentration of antibiotics: 100 µg/ml	
121.01	1.077
152.58	1.358
183.14	1.630
Concentration of antibiotics: 200 µg/ml	
100.56	0.895
175.28	1.560
155.05	1.380
Concentration of antibiotics: 1 mg/ml	
91.7	0.817
155.05	1.380
168.08	1.496

significant. The F value obtained also depicts that the variance ratio between the given values is significant (Table 10).

The results obtained by the SPSS logistics software showed that the frequencies of the values obtained at different compositions of serum protein are significant (Tables 11 and 12) (Graph 2 and 3).

SERUM COLOR

The colour of chick, pigeon, dove and tortoise samples produced is orange red whereas that of toad and lizard is very faint yellow. The serum colour of other vertebrates is colourless (Table 1).

CONCLUSION

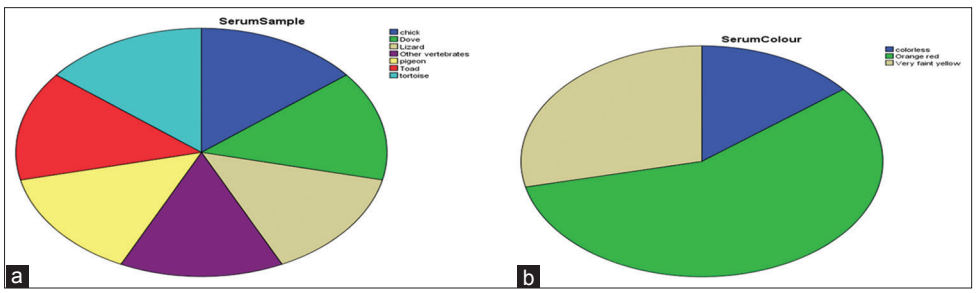
Table 13 shows different rates of optical density of proteins present in chick blood serum in addition of different concentrations of the two antibiotics, namely, ampicillin and chloramphenicol. The results obtained by addition of ampicillin and chloramphenicol antibiotics in 10, 20, and 30 mg/l panoply that, as the concentration of serum increases, the concentration of proteins (without antibiotics) increases significantly. However, it is not observed very linearly in case of 1 mg/ml ampicillin and 200 µg/ml chloramphenicol antibiotic. This clearly indicates the validation of the Beer–Lambert's law (Table 13). The principle of spectrophotometry and Beer– Lambert's law help us to know the absorbance pattern of proteins in the serum and hence their respective concentrations. This is one of the experiments used in the laboratories at the commercial level to prepare different antibiotics. The rise and fall in the absorbance rate of proteins after addition of different antibiotics represent the increase and decrease in the concentration of proteins, respectively. This shows that every antibiotic acts at a particular concentration on the protein of the serum. Therefore, proper doses of antibiotics are recommended by the doctors.

Table 9: One-way ANOVA results of different concentrations of ampicillin antibiotic

One-way ANOVA results					
Source	**df**	**SS**	**MS**	**F**	**p value**
Concentration of protein in serum with respective absorbance					
Without antibiotics					
Treatments	1	22371.041	22371.041	173.8343	0.0034
Error	4	514.767	128.692		
Total	5	22885.808			
With antibiotics: For ampicillin					
Concentration of antibiotics: 100 µg/ml					
Treatments	1	26609.497	26609.497	29.7590	0.0408
Error	4	3576.669	894.167		
Total	5	30186.166			
Concentration of antibiotics: 1 mg/ml					
Treatments	1	36892.689	36892.689	1294.2044	0.0002
Error	4	114.024	28.506		
Total	5	37006.714			

Table 10: One-way ANOVA results of chloramphenicol antibiotic on chick serum

For chloramphenicol					
Source	**df**	**SS**	**MS**	**F**	**p-value**
Concentration of antibiotics: 100 µg/ml					
Treatments	1	34150.934	34150.934	70.7648	0.0124
Error	4	1930.391	482.598		
Total	5	36081.325			
Concentration of antibiotics: 200 µg/ml					
Treatments	1	30395.996	30395.996	40.6989	0.0268
Error	4	2987.400	746.850		
Total	5	33383.396			
Concentration of antibiotics: 1 mg/ml					
Treatments	1	28172.272	28172.272	33.7470	0.0345
Error	4	3339.233	834.808		
Total	5	31511.505			



Graph 2: (a and b) The different results of the chick serum comparison of different vertebrates by SPSS logistics software

Table 11: The different frequencies obtained by the SPSS software of composition of proteins of different vertebrates

Statistics	Mobility	Sample	pH	Ionic strength	Dilution
N					
Valid	6	6	6	6	6
Missing	0	0	0	0	0
Mean	4.7333		7.3000	0.1500	
Median	4.5500		7.3000	0.1500	
Mode	3.80[a]		7.30	0.15	

[a]Multiple modes exist. The smallest value is shown

Mobility	Frequency	Percentage	Valid percentage	Cumulative percentage
Valid				
3.80	1	16.7	16.7	16.7
4.00	1	16.7	16.7	33.3
4.40	1	16.7	16.7	50.0
4.70	1	16.7	16.7	66.7
5.30	1	16.7	16.7	83.3
6.20	1	16.7	16.7	100.0
Total	6	100.0	100.0	

Sample	Frequency	Percentage	Valid percentage	Cumulative percentage
Valid				
Cat	1	16.7	16.7	
Chick	1	16.7	16.7	16.7
Dog	1	16.7	16.7	33.3
Human	1	16.7	16.7	50.0
Monkey	1	16.7	16.7	66.7
Pigeon	1	16.7	16.7	83.3
Total	6	100.0	100.0	100.0

pH	Frequency	Percentage	Valid percentage	Cumulative percentage
Valid				
7.30	6	100.0	100.0	100.0

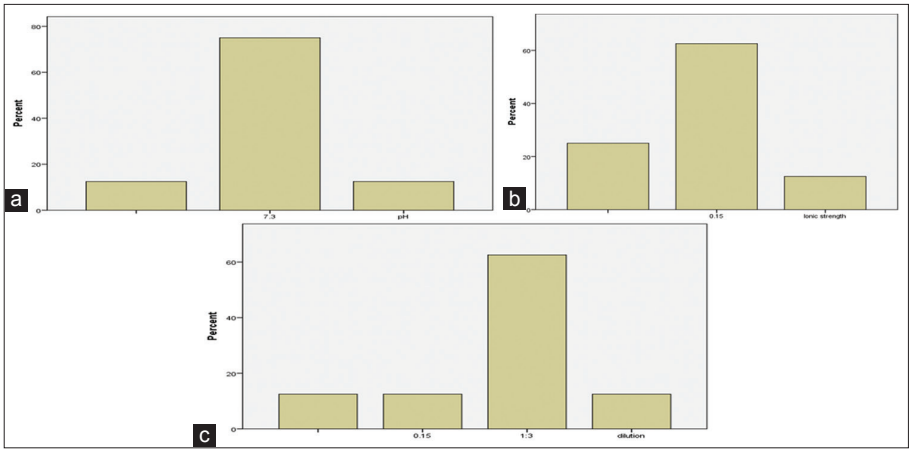
Ionic strength	Frequency	Percentage	Valid percentage	Cumulative percentage
Valid				
0.15	6	100.0	100.0	100.0

Dilution	Frequency	Percentage	Valid percentage	Cumulative percentage
Valid				
1:3	6	100.0	100.0	100.0

Table 12: The different results of chick serum comparison of different vertebrates by SPSS logistics software

Serum sample	Frequency	Percentage	Valid percentage	Cumulative percentage
Valid				
Chick	1	14.3	14.3	14.3
Dove	1	14.3	14.3	28.6
Lizard	1	14.3	14.3	42.9
Other vertebrates	1	14.3	14.3	57.1
Pigeon	1	14.3	14.3	71.4
Toad	1	14.3	14.3	85.7
Tortoise	1	14.3	14.3	100.0
Total	7	100.0	100.0	

Serum color	Frequency	Percentage	Valid percentage	Cumulative percentage
Valid				
Colorless	1	14.3	14.3	14.3
Orange red	4	57.1	57.1	71.4
Very faint yellow	2	28.6	28.6	100.0
Total	7	100.0	100.0	



Graph 3: (a-c) The different frequencies obtained by the SPSS software of composition of proteins of different vertebrates

Table 13: Summary of the results obtained by the concentration of serum protein (without antibiotics) and concentration of protein with ampicillin and chloramphenicol

Concentration of serum (mg/l)	Concentration of protein (without antibiotics)(mg/l)	Concentration of protein (with antibiotics) (mg/l)					
		Ampicillin			Chloramphenicol		
		100 µg/ml	200 µg/ml	1 mg/ml	100 µg/ml	200 µg/ml	1 mg/ml
10	111.3	86.40	86.40	164.83	121.01	100.56	91.7
20	116.9	150.56	150.56	150	152.58	175.28	155.05
40	141.46	166.2	166.2	159.88	183.14	155.05	168.08

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