ISOLATION AND IDENTIFICATION OF ESCHRESIA COLI FROM URINARY TRACT INFECTION IN QUETTA, BALOCHISTAN

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Abstract

This study was aimed to isolate and identify the frequency of E .coli causing urinary tract infection in human beings. Total of one hundred (100) urine samples were collected from patients in sterile container visiting Bolan medical Complex, Quetta. The pellet was collected after centrifugation and was inoculated onto Eosine Methylene blue Agar and Mac Conkey agar plates. After confirmation of the organism through staining and morphology, a series of biochemical tests viz, Indole, methyl red, Voges proskaur, Citrate utilization and catalase test were performed. E coli were isolated from 72 samples, indicating its great share in causing the urinary tract infection.

Keywords: *E .coli, Eosine Methylene blue, MacConkey agar, centrifugation.*

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INTRODUCTION

Urine is a variable but generally good culture medium for bacteria. The rate of extent of microbial growth depend on the pH, tonicity, concentration of urea and presence of dietary-derived organic acid (kunin *et al,* 1992).Urinary tract infection (UTI) is one of the commonest bacterial infections seeking treatment in clinical practice. Although a variety of etiology is involved with UTI, *E. coli*•and other coliforms account for large majority of naturally acquired urinary tract infections (Mogoha,1997). Over half (53%) of all women and 14% of men experience at least one urinary tract infection (UTI) in their lifetime (Griebling, 2005). Urinary tract infections are broadly categorized into three main types based on the site of infection bladder (cystitis), urethra (urethritis) and kidney (pyelonephritis). Usually it is caused by a range of Gram-negative and Gram-positive organisms, including *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Providencia stuartii, Staphylococcus epidermidis, and Enterococcus faecalis* (Hull *et al* .,1998).

In many cases, the bacteria that cause these infections are resistant to multiple antibiotics, and thus they pose a serious threat to the safety and proper functioning of health care facilities. The urinary tract is among the most common sites of bacterial infection in humans and *E. coli* is by far the most common species infecting this site, accounting for more than 80% of community-acquired infections (kunin, 1987).

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped, flagellated, motile, oxidase negative, facultative anaerobe and is classified under the family Enterobacteriaceae (Buxton and Fraser, 1977). Epidemiological studies have shown that bacterial adherence to mucosal surfaces is a critical virulence factor for UTI. *E. coli* express different types of adhesion factors, as P and type 1-fimbriae, thin hair like structures, which mediate binding to receptors or receptor epitopes present on the uroepithelium (Leffler and svanborg,1981). *E. coli* is genetically the most versatile bacteria and

is the normal constituent of the animal intestinal microflora living as commensel.This study was designed to isolate the E coli from urinary tract infection in human.

MATERIALS AND METHODS

Total of one hundred (100) urine samples were collected in sterile containers from patients suffering from urinary tract infection in Bolan medical College Complex Hospital, Quetta. The sample were centrifuged at 3000 rpm for 5 minutes and the pellet was inoculated onto freshly prepared Eosine Methylene blue agar and MacConkey agar Plates. The inoculated plates were kept at 37 ^{0}C for 24 hours for incubation as described by (Lynn *et al.*, 1998).

Gram staining characters

A heat fixed thin smear was prepared with the help of wire loop of the inoculated coloy on pre cleaned glass slide. The slide was stained for gram staining as proposed by (Cappuccino and Sherman, 2007). Small rods of pink color were observed.

Biochemical tests

A series of biochemical tests were carried out to identify the E coli from collected urine samples.

Indole production test

Tryptophan broth was dispensed in test tubes @ 05 ml and was sterilized by autoclaving at 121 ^{0}C at 15 pound pressure for 15 minutes. The tube was inoculated with the loopful culture test organism and incubated aerobically at 37^{0}C for 24 hrs. 0.3 ml of kovac's reagent were added and dark pink color ring were observed within 5-10 seconds for positive organisms.

Methyl Red (MR) and Voges Proskaur (VP) test

The MR-VP medium was prepared @ 10 ml in each test tube and autoclaved by sterilization. Loopful cultures of the test organism were added. After incubation for 24 hrs aerobically the inoculated tubes culture were divided into two plates. 2-3 drops of Methyl red was added in one of the tube. The presence of Red coloration was the indication of positive test. While the second tube was incubated with equal quantity of Omera's Reagent and incubated for 3-4 hrs at 37 ^{0}C and the results were recorded.

Citrate utilization test

Simmon citrate agar slants were prepared and were inoculated with the loopful of test culture. The tubes were incubated at 37 ^{0}C for 24 hrs. The change in colour of the slants from green to blue was the indication of Positive test.

Catalase test

This test was performed by transferring the small colony onto clear glass slide and mixed with 2-3 drops of H_2O_2 (Hydrogen peroxide). The results were recorded within seconds in the form of bubble formation in case of positive tests.

Motility test

The Motility medium was prepared in 3-4 ml in test tubes, autoclaved and stabbed with test organism. The tubes were incubated at for 24 hrs and the results were recorded in the form of hassiness in positive cases as described by (Arora, 2003).

RESULTS AND DISCUSSION

Out of total of 100 urine samples, Eighty eight (88) samples were positive for Eschresia coli, as the culture growth were present in both Mac Conkey and Eosine methylene Blue (EMB) agar plates. Morphologically the colonies were circular and convex. The pink colour growth was present on MacConkey while EMB plates exhibited Metallic shine colour. Similarly on gram staining the small sized gram negative bacilli were seen. Biochemically the E. coli positive samples were positive in motility medium, Indloe and Methyl red test while were found negative for Voges Proskaur (VP) , Catalase, and Citrate utilization test (table 1).

These results are in accordance with (Buxton and Fraser, 1977), who also reported E. coli with same biochemical results. These results clearly indicate that E. coli are the predominant cause of urinary tract infection. Although a variety of etiological agents are responsible for UTI, but the most common cause is E. coli in addition to other coliforms. It is also the frequent cause of nosocomial infection (Magoha, 1997). This study also reflected that UTI were more common in Female than male, as ¾ of the patients were

female. These findings corroborate with (Ibeawuchi and Mbata, 2002) who also reported more UTI cases in female than male, principally owing to certain physical factors and anatomical structure.

In conclusion E coli is the major culprit in the urinary tract infection that may complicate the infection if not properly treated. There is dire need to collect huge number of samples and to determine their antimicrobial susceptibility and sensitivity to different antibiotics that may be helpful in proper treatment of the patients.

Table-1- Biochemical Tests for isolation of E.coli from urine samples.

Organisms	Indole	MR	VP	Citrate Utilization	Catalase	Motility	Gram Staining
Eschresia coli	+	+	-	-	-	+	-

MR= Methyl Red
VP= Voges Proskaur

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CHARACTERIZATION OF INGAN BY MEANS OF C-V MEASUREMENT OF RESPECTIVE LIGHT EMITTING DIODE (LED) BY DLTS

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Abstract

Indium Gallium Nitride (InGaN) has been characterized by means of capacitance spectroscopy. The capacitance voltage measurement of respective schottky diode is performed by standard method available in our DLTS setup. The capacitance voltage measurements of InGaN obtained at various temperatures under the same reverse biasing conditions for material. From these measurements the following parameters were evaluated: The doping concentration of InGaN at 280K was calculated as 1.0999×10^{15} cm^{-3} and showed no significant temperature effect. The built in potential calculated for InGaN was 3.35V at 280K and Its value gradually decreased with increase in temperature. The depth profile of InGaN remained consistent and showed no change as the temperature varied from room temperature to lower values. Comparison of the data with the literature showed that all the samples were affected by native and/or intrinsic point defects developed during growth or metallization process.

Keywords: *Semiconducting Indium Gallium Nitride materials, I-V characteristics, C-V characteristics, schottky diode*.

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INTRODUCTION

Indium gallium nitride (InGaN, InxGa1-xN) is a semiconductor material made of a mix of gallium nitride (GaN) and indium nitride (InN). It is a ternary group III/group V direct bandgap semiconductor. Its band gap can be tuned by varying the amount of indium in the alloy. The ratio of In/Ga is usually between 0.02/0.98 and 0.3/0.7. Indium gallium nitride is the light-emitting layer in modern blue and green LEDs and often grown on a GaN buffer on a transparent substrate as, e.g. sapphire or silicon carbide. It has a high heat capacity and its sensitivity to ionizing radiation is low (like other group III nitrides), making it also a potentially suitable material for solar cell arrays for satellites. The wavelength emitted, dependent on the material's band gap, can be controlled by the GaN/InN ratio, from near ultraviolet for 0.02In/0.98Ga through 390 nm for 0.1In/0.9Ga, violet-blue 420 nm for 0.2In/0.8Ga, to blue 440 nm for 0.3In/0.7Ga, to red for higher ratios and also by the thickness of the InGaN layers which are typically in the range of 2-3 nm (Wikipedia).

MATERIALS AND METHODS

Sample preparation

The commercially available blue light emitting diode of InGaN with diameter of 0.5mm is analyzed in this research.

Capacitance-Voltage C-V Measurements

The C-V measurements are necessary to determine the diode quality as well as to determine depth profile. At different

temperatures capacitance voltage measurements were taken for various materials. From the C-V measurement we find the carrier concentration, built-in-potential and depth profile: Doping concentration 'N_d', Built-in potential 'V_{bi}' and Depth profile 'depth vs. N_d,

$$N_d = 2/[q\varepsilon_m * \text{slope}] \quad (1)$$

$$V_{bi} = [\text{intercept} * q\varepsilon_m N_d]/2 \quad (2)$$

$$Nd = [2(V_a + V_{bi})]/ [q\varepsilon_m d/dv(A^2/C^2)] \quad (3)$$

And

$$D(C) = \varepsilon_m A/C$$

Where

V_a = Applied voltage, V_{bi} = Built-in potential,
q = Electron charge,
ε_m = Relative permittivity of the material, D = Depth

RESULTS AND DISCUSSION

Capacitance-Voltage C-V Measurements of InGaN

Measurements of the C-V characteristics over a voltage range of -9V to 3.9V, the typical C-V characteristics of InGaN (commercially used blue LED) at different temperature 280K to 167K are shown in Figures 1 to 10. In the graphs of Voltage (V) and $(A/C)^2$ the straight lines is taken as the theoretical linear fitting of the curves. The graphs of depth profiles have taken between depth and doping concentration shown in Figures 11 and 12. Using equations (Wikipedia) and (Rafal, 2005) and depth profile using equation (Tzer-En Nee *et al*.,2005) we have calculated the following parameters shown in table below.

Temperature (T) K	Doping Concentration (N_d) cm^{-3}	Built in potential (V_{bi}) V
280	1.00999×10^{15}	3.34
260	1.0777×10^{15}	3.46
220	1.08985×10^{15}	3.85
200	1.1483×10^{15}	4.38
167	1.1971×10^{15}	6.35

Table: 1 Parameters calculated from CV measurements of InGaN.

From table 1 the values of doping concentration (N_d) remained almost constant with the variation of temperature. The calculated value of N_d at temperature 280K was $1.00999\times10^{15}cm^{-3}$ but built-in potential (V_{bi}) varies with the change of temperature. The calculated value of Vbi was 3.34V at 280K which increased gradually at lower temperature value and determined as 6.35V at 167K.(Tzer-En Nee *et al*.,2005)

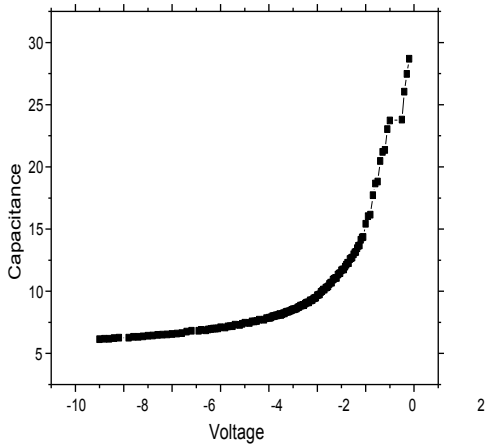

Figure 1: The graph between C-V of InGaN at T=280K.

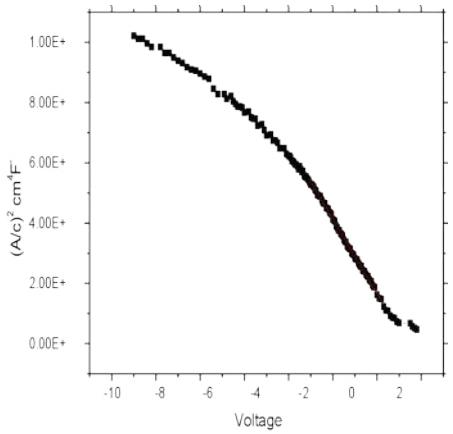

Figure 2: The graph between V and $(A/C)^2$ of InGaN at T=280K.

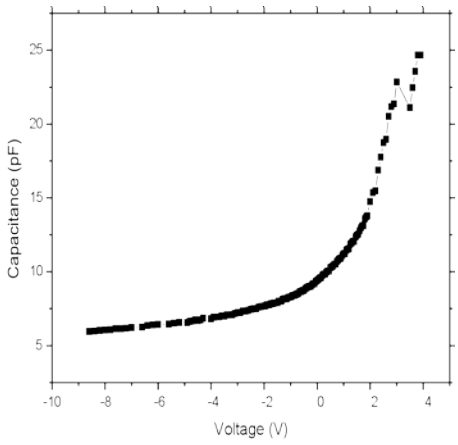

Figure 3: The graph between C-V of InGaN at T=260K

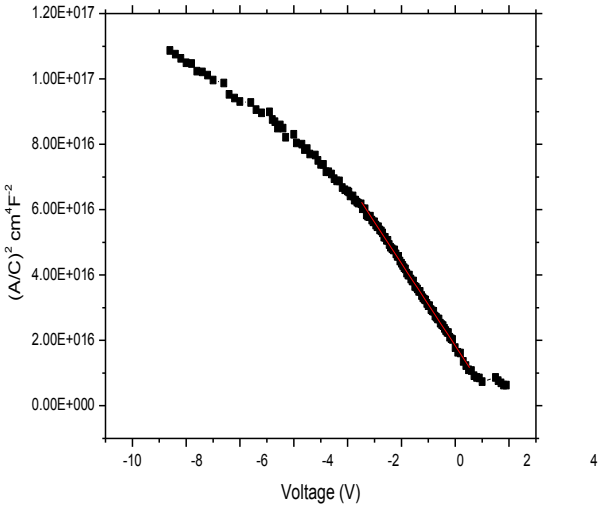

Figure 4: The graph between V and $(A/C)^2$ of InGaN at T=260K.

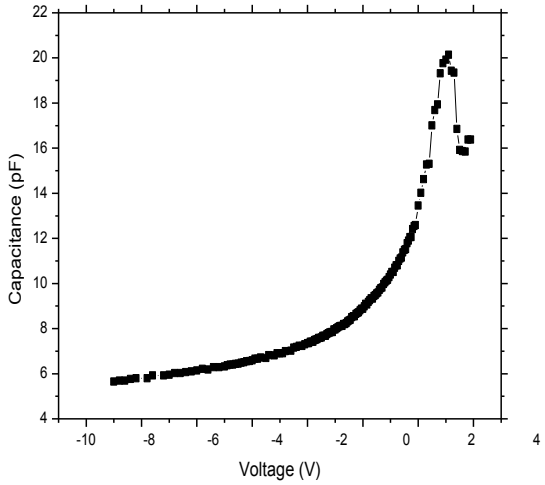

Figure 5: The graph between C-V of InGaN at T=220K .

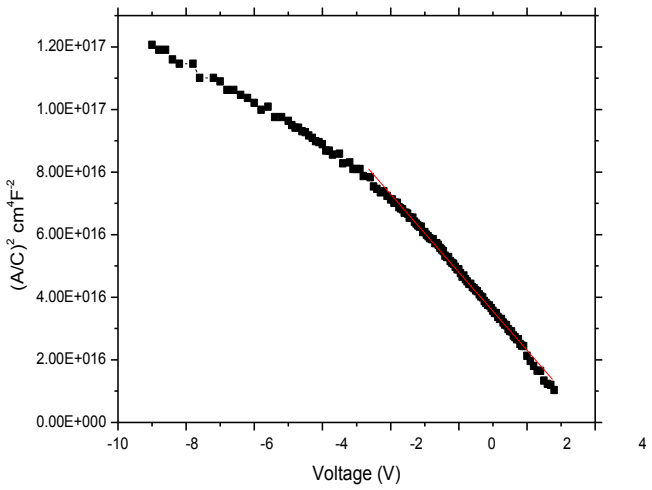

Figure 6: The graph between V and $(A/C)^2$ of InGaN at T=220K.

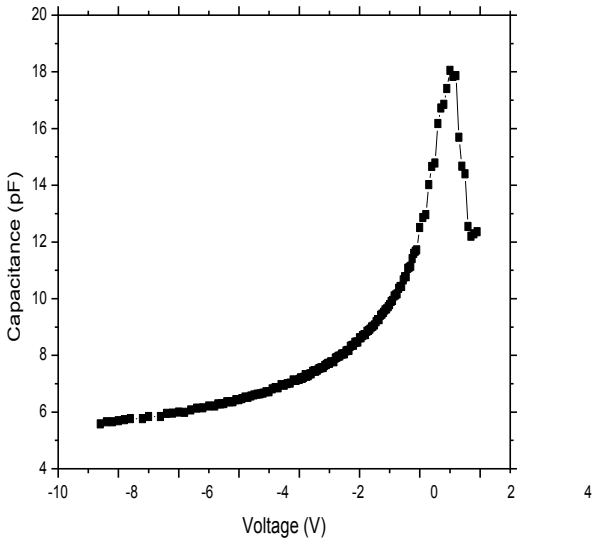

Figure 7: The graph between C-V of InGaN at T=200K

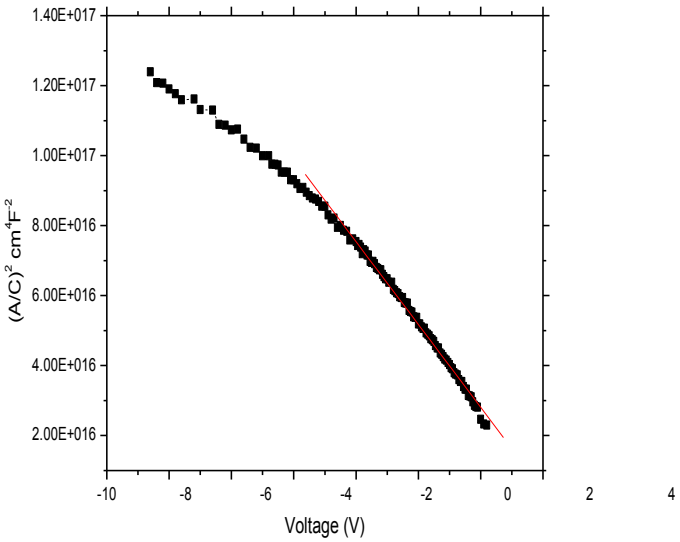

Figure 8: The graph between V and $(A/C)^2$ of InGaN at T=200K.

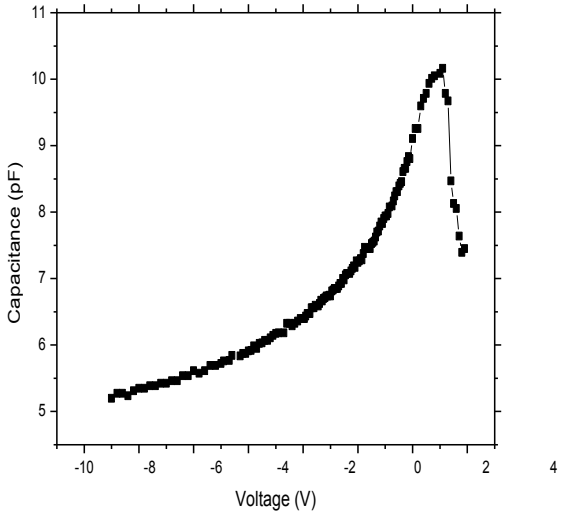

Figure 9: The graph between C-V of InGaN at T=167K .

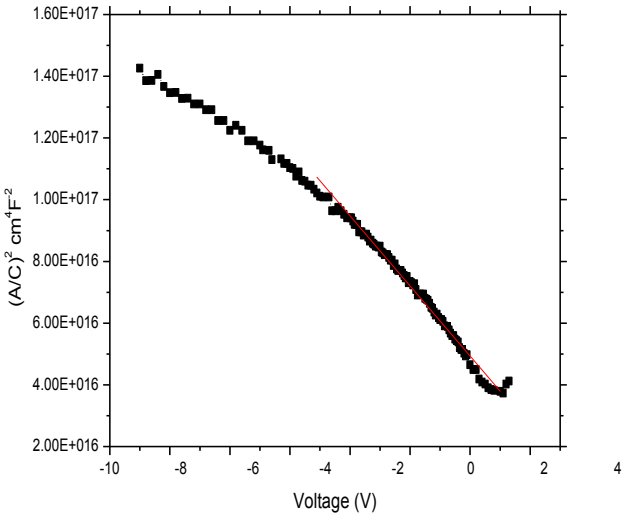

Figure 10: The graph between V and $(A/C)^2$ of InGaN at T=167K.

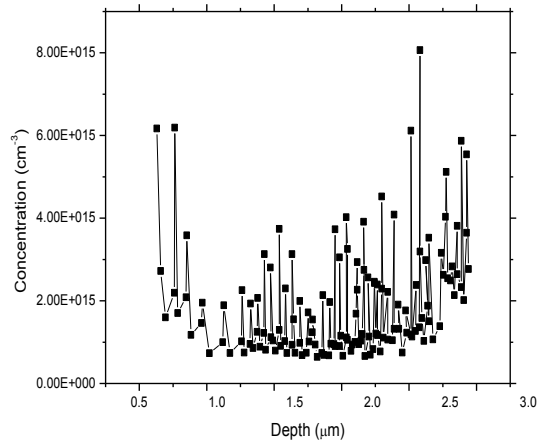

Figure 11: Depth profile of InGaN at temperature 280K .

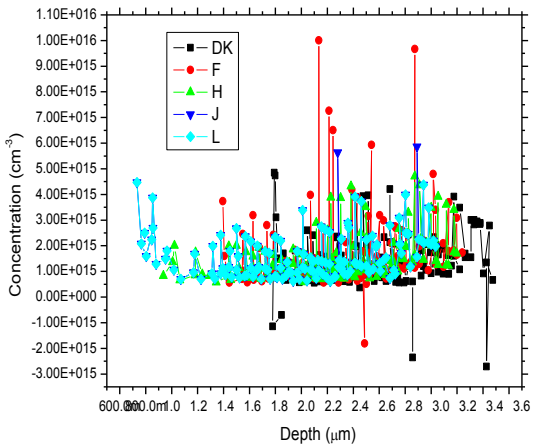

Figure 12: Depth profile of InGaN at various temperatures.

CONCLUSION

We studied the characterization of InGaN by means of current and capacitance spectroscopy. The I-V characterization was performed to evaluate the rectifying behavior of the diode. The ideality factor (n), reverse saturation current (I_s) and barrier height (φ_B) of the diodes at various temperatures were evaluated. Doping concentration (N_d), buit-in potential (V_{bi}) and depth profile were determined by C-V measurements. Sample wise detail is given as under:-

I-V and C-V measurements of InGaN (commercially available blue light emitting diode) were taken at 305, 280, 260, 230, 220, 210, 200 and 170K. The calculated values of n, I_s and φ_B at R_T were 8.83, 8.22×10^{-11}A and 0.851 V respectively. N_d and V_{bi} at 180K were $1.00999\times10^{15}cm^{-3}$ and 3.35eV respectively. By the variation of temperature, investigated I-V characteristics show that the conduction in a diode is controlled by thermionic field emission. C-V characteristics show that V_{bi} depends on temperature while, N_d approximately remains constant with change in temperature.

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