Effect of Some Solvents on Spectroscopic Properties of Rhodamine 3GO Dye.

*Ali H. Al-Hamdani, *Slafa Ibrahim, **Raja Abdul- Ameer Madlool and ***Hussein A. Al-Hamdani

* Energy and Renewable Energies Technology Center, University of Technology, Baghdad, Iraq.
**Department of Physics, College of Science,University of Kerbala , Kerbala, Iraq.
***Department of Electrical and Electronics Engineering, College of Engineering, University of Kerbala,Iraq.

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الخلاصة

تم في هذا البحث دراسه تأثير بعض المذيبات (الإيثانول والميثانول، -2بروبانول، الأسيتون، وخلات الإيثيل) على طيفي الامتصاص والفلورة لصبغة الرودامين (3GO). أظهرت النتائج أن هناك زيادة في الكفاءة الكمية للإمتصاص مع زيادة الاستقطاب النسبي من المذيب وتناقصها مع زيادة لزوجة المذيب. يتم إزاحة طيف الفلورة نحو الطول الموجي الطويل (الأحمر). وتبين أن قيم ذروة الامتصاص لصبغة الرودامين في المذيبات اعلاه تقع بين الاطوال الموجيه(525-530) نانومتر، في حين أن قيم ذروة الفلوره بين(551.5-558.5) نانومتر وان العمر الزمني لطيف الفلورة يزداد بزيادة الكفاءة الكمية.

الكلمات المفتاحية

صبغة الرودامين (3OG)، الكفاءة الكمية للإمتصاص، العمر الزمني لطيف الفلورة.

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However, all A.hydrophila isolates were totally sensitive 100% to the Nalidixic acid, Tetracycline and Gentamycin. The obtained results of this study is almost in agreements with [35] in terms of resistance rates for Cephalothin ,Amoxicillin/sulbactam,Ampicilin,Cefotaxime,Imipenem,ceftriaxone ,Trimethoprim/sulfamethoxazol, Tobramycin and Chloramphenicol, and also in terms of its sensitivity to Nalidixic acid. So the finding of this study agreed with the results that obtained from both [35,36] as A.hydrophila was isolated from patients children whom suffering from diarrhea.While the results disagreed with [35] results in terms of A.hydrophila susceptibility to Tetracycline, Gentamycin, Ciproflaxacin, Amikacin and Norfloxacin.

On the other hand, there are similarities in the results that obtained from the current study with [11] finding in terms of A.hydrophila isolates susceptibility to Amoxicillin, Ampicilin, Cephalothin, Gentamycin and Nalidixic acid, but it was differ in its susceptibility to Tetracycline, Chloramphenicol, Tobramycin, Ciprofloxacin, Trimethoprim and Ceftriaxone.

In fact, the key word behind the differences in A.hydrophila resistance to antibiotic is that most of aeromonads produce an inducible chromosomal β – lactamase with activity against a wide variety of β-lactam antibiotics [37], moreover, antibiotics resistance to chloramphenicol, streptomycin , tetracycline, cefoxitin, cephalexin , erythromycin, furazolidone, and sulfathiazole is mediated by plasmids [38]. [39] observed that the treatment of bacterial-associated diarrhea become more intricate by the frequency of antimicrobial resistance in many pathogens. Data concerning antimicrobial resistance for A. hydrophila have been varied.

3.4. The index of multiple antibiotic resistances :-

In this study the index of multiple antibiotic resistances (MAR) has been calculated for A.hydrophila isolates and result was 0.26 for all isolates, and was indicative to wide antibiotics usage. As it was presented by [40,41] reports whom determined MAR for a number of A. hydrophila strains.

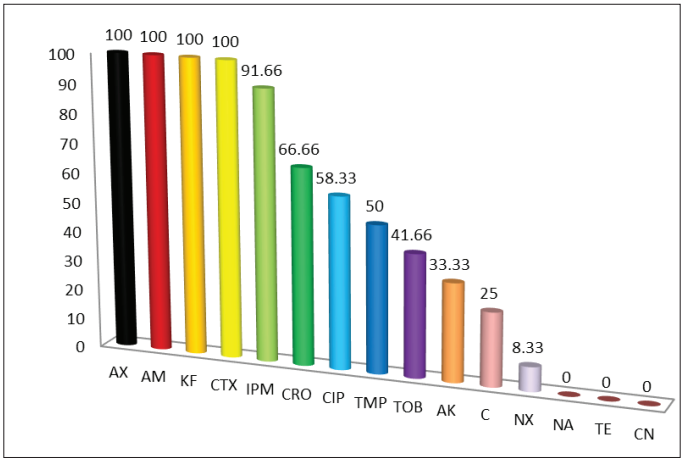

Fig. (2): Antibiotics susceptibility of A.hydrophila isolates.

3.2. Virulence factors

Number of virulence factors of A.hydrophila isolated from patients children whom suffering from diarrhea disease were detected. The results revealed that all A.hydrophila isolates were positive to the β-heamolysin. The results of this study in agreements with [5,2] whom described that; β-hemolysins as an important bacterial virulence factors which promoting channel formation leading to cell death. As well as, all the isolates showed positive results to phospholipase test. The phospholipase produce a precipitate zone when grown on egg yolk medium according to [24]. This is agreed with [25,26] whom indicated that phospholipases play an important role in pathogenesis of A. hydrophila.Furthermore, all isolates give a positive results to protease test when was cultured on skim milk media. Another study noticed that A.hydrophila has the ability to produce protease enzyme that act to hydrolyze protein [27]. Additionally, A.hydrophila isolates presented positive results to lipase test that able to hydrolyze fat. Also [28] indicated that A. hydrophila is known to secrete lipases and have been suggested that lipases may supply nutrients and contribute virulence factors by react with human leukocytes or by affecting many immune system functions through free fatty acids generated by lipolytic activity [29].

In addition, all isolates showed positive results to the biofilm formation. In their reports [30,31] presented that the persistence of A. hydrophila in biofilms within water distribution systems, as well as, their multiple resistance (R), after isolated from these utilities were for a long time undervalued, while these aspects are signifying an important risk for the public health [32,33].Moreover, all isolates of A.hydrophila exhibited capsule. The results of this study are also agreed with the finding of [33] which is the role of A. hydrophila capsule polysaccharide as virulence factor since most motile strains.Likewise, A.hydrophila isolates appeared a positive result to motility after inoculated on semisolid media and that leads to disseminating of growth out of the stab line after indication. This result agreed with [4] who showed that A. hydrophila composed of motile isolates which grew well at (35 to 37)°C and was associated with a variety of human infections .Table (4).

Table (4): Virulence factors of A.hydrophila.

No.	Type of virulence factors	Result
1.	Haemolysin	β-type
2.	Phospholipase	+
3.	Lipase	+
4.	Protease	+
5.	Biofilm formation	+
6.	Capsule	+
7.	Motility	+

3.3. Antimicrobial Susceptibility

The antibiotics sensitivity of A.hydrophila isolates were investigated according to [19,34]. The results that obtained from this study(Fig 2) revealed that all A.hydrophila isolates were gave 100% resistant to the Amoxicillin, Ampicilin, Cephalothin and Cefotaxime, while A.hydrophila isolates were showed resistance rates 91.66%, 66.66%, 58.33%, 50%, 41.66, 33.33%, 25% and 8.33% to Imipenem, ceftriaxone, Ciproflaxacin, Trimethoprim, Tobramycin, Amikacin, Chloramphenicol and Norfloxacin respectively.

kligler iron agar (Alk/Acid). Where the red colour (Alkaline) was appeared in the top of the slant agar and the yellow colour (acidic) was appeared in the bottom of slant agar with no H_2S production and this is in agreement with [11]

In addition, A.hydrophila gave variable results to vogues-proskauer and lysine decarboxylase test, the presented results are agreed with [21,22] in terms of positive results, while, it is agree with [12,23] in the negative results. All of A.hydrophila isolates gave negative results to string test (sodium deoxycholate). This test considered to be a differential test between A.hydrophila and V.cholera [21]. In addition PCR technique was used to confirm identify A.hydrophila and all isolates have given a positive result for 16Sr RNA gene [11] Fig (1).

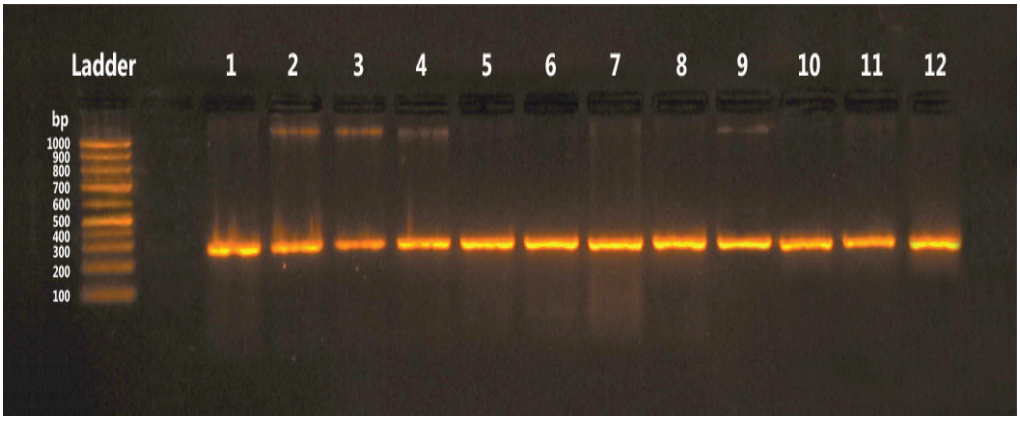

Fig. (1): Agarose Gel Electrophoresis of PCR Amplified of 16S rRNA Gene (300) bp of Aeromonas hydrophila Isolates for 1 hr at (80) Volt. Lane 1 DNA Marker (100bp Ladder). Lane 2,3,4,5,6,7,8,9,10,11,12,13 Amplify of 16Sr RNA Gene in A. hydrophila

Table (3): Biochemical tests of Aeromonas hydrophila isolates.

No.	Type of test	Result
1.	Oxidase test	+
2.	Catalase test	+
3.	Indol test	+
4.	*MR test	+
5.	VP.test	±
6.	Citrate test	+
7.	Kligler iron test	Alk\A
8.	Gelatin liquefaction	+
9.	String test	_
10.	Lysine decarboxylase	±

*MR: methyl red VP: vogues-Proskauer Alk\A: Alkaline\acid
+: positive result -: negative result ±: variable result.

3) min, after that discard the stain and wash the slide by (20%) CuSO4.7 H2O (don't use water) and examined under microscope. Capsule appears as a clear zone around the body of the bacteria which appeared blue [18].

2.2.5. Motility

To prepar motility medium (4) gm of agar add to (100) ml of nutrient broth after that completed up to (1000) ml with D. W. Then it has been sterilized by autoclave at (121) ^{o}C for (15) minutes. After that the motility medium has been distributed in tubes then used to detect bacterial motility. Then the tubes that were contained semisolid media inoculating with bacteria by a stabbing method and has been incubated at (37)^{o}C for (24-48) hr. A spread growth out of stab line indicate positive result appeared when the was [12].

2.3. Antimicrobial Susceptibility

According to [19] the antibiotics and its standard inhibition diameters was used to detect antimicrobial susceptibility. To prepar the inoculums (3-5) isolated colonies grown on nutrient agar plate has been added to (5) ml of sterile normal saline and then compared with (1.5X 108) cl/ml McFarland standard tube. To obtain inoculums from the bacterial suspension A sterile swab has been used in this technique . After that the inoculums has been streaked on a Mueller-Hinton agar (MHA) plate then left to dry. The antibiotics discs has been placed on the surface of the medium at equally spaced intervals with flamed forceps or a disc applicator then it was incubated for (24) hours at (37)^{o}C. To determine the sensitivity or resistance of the organism to each antibiotic the inhibition zones were measured and compared with the zones of inhibition determined by the Clinical Laboratory Standards Institute [12]

2.4. Determine the multiple antibiotic resistance index (MAR)

The multiple antibiotic resistance index was calculated for each isolate separately and determined by divided the number of antibiotics, on which the isolate is resistant over the total number of antibiotics, towards which the isolate susceptibility was checked. The MAR index, that was higher than 0.2 (>0.2) identifies bacteria isolated from objects with higher risk of contamination, whereas antibiotics was often used. The MAR index (≤ 0.2) identifies strains from the environment, however, antibiotics are not used at all or are rarely used [20].

$$\text{MAR index} = \frac{\text{Number of antibiotics which the isolate is resistant}}{\text{Number of antibiotics which the isolate susceptibility has been checked}}$$

3. Results and Discussion

3.1. Samples Collection and Identification of Aeromonas hydrophila

In this study (12) isolate obtained from (294) diarrheic stool samples that were collected from Al-Muthana public health laboratory. To confirm initial diagnosis of bacteria a manual biochemical tests were used Table (3) have shown that A.hydrophila was presented a positive result for each of oxidase, catalase , methyl red, gelatin liquefaction , simmone citrate, and Indole. The finding results of this study are almost simillar the other researchers reports [21,22]. Moreover, A.hydrophila has the ability to ferment glucose on

According to information of manufacturing company (Master mix, Geneaid/Taiwan) PCR mixture solution and PCR Program conditions was listed in Table (2) [11]. In first well on (1%) agarose gel ten ml standard molecular weight of DNA ladder (marker) was loaded and each well has been loaded with (10) ml of PCR product (DNA sample). Electrophoresis runs at (80) volt/cm for 1hr.

Table (2): Amplification conditions

Steps	Temperature	Time	No. of cycles
Initial denaturation	94 °C	3 min	30 cycle
Denaturation	94 °C	30 sec	
Annealing	52 °C	30 sec	
Elongation	72 °C	30 sec	
Final elongation	72 °C	10 min	

2.2. Investigate of Some Virulence Factors

2.2.1. Protease activity assay

Protease medium was prepared by the following solutions:-

1. Add10g from skim milk to (90) ml of D.W and completed to the (100) ml, then, gently heated at (50)˚C, and without autoclave.

2. Add (2)g of agar powder to the (100) ml of D.W and mixed thoroughly, then autoclaved, and cooled to (50-55)˚C.

3. Preparation medium:- (100) ml of solution (1) has been mixed with (100) ml of solution (2) directly. Then was poured into sterile Petri dishes and by streaking method a single colony was cultured on skim milk agar and was incubated at (37) ^{0}C for (24- 48) hr, and a cleared hydrolysis zone indicates of positive test [15].

2.2.2. Phospholipase activity assay

Phospholipase medium was prepared by dissolving (1)g of NaCl in (100) ml of nutrient agar. Then sterilize by autoclave and was cooled to the (50) ^{0}C. After that in sterilized conditions one egg yolk has been added to the mixed medium and mixed well. Then, the mixed medium has been poured into the sterilized Petri dishes and inoculated with a single colony and incubated for (24-72) hr at (37)^{0}C. As a positive result the color of precipitation zone around the colonies should be change from white to brown color [16].

2.2.3. 1%Tween20 media of lipase activity assay

This media was prepared according to the [17] as follows:- (1) ml of (1%) Tween (20) was added to (100) ml of nutrient agar after that sterilized by autoclaving and poured in to sterilized Petri dishes and inoculated with a single colony and incubated for (1-5) days at (37)°C. after added the CuSO4 .5H2O regent to incubated culture, the turbid zone around colonies with change to blue color, indicates a positive result.

2.2.4. Capsule test

A colony of young bacterial isolates was mixed with drop of normal saline on clean slide and late smear to dry at room temperature (don't use flame). Then crystal violate stain was added to (2-

1. Introduction:

Aeromonads are microorganisms living water, and is often found in chlorinated and non-chlorinated water and bottled drinking water supplies in bottles. As it has been isolated from food . Therefore, food and water from potential sources of human infection [1]. Some species of aeromonads cause opportunistic infections in humans and diseases of aquatic animals [2]. Aeromonas is mostly known as an enteric pathogen. A strong correlation between gastroenteritis and Aeromonas species has been shown in children, adults who are older than (60) years and in cases of 'traveler's diarrhea [3]. In fact, the three most common human infections caused by Aeromonas species are gastrointestinal infection, skin and soft-tissue infection, and bacteremia in immunocompromised individuals[4]. Aeromonas hydrophila are most often the advantage of being caused intestinal and extraintestinal human infections [1]. The potential virulence of aeromonad that lead to the pathogenicity include, cell structural (cell-associated structures) outermembrane proteins, lipopolysaccharides, flagella and pili, a type III secretion system (T3SS) acting as adhesion structures, and extracellular factors such as enzymes and toxins [5,6]. A. hydrophila virulence factors that cause diarrhea which include intestinal cytotoxic and intestinal cytotonic [7 ,8,9]. As well as multiple drug resistance occurred more in A. hydrophila than other species of Aeromonas spp and that isolates from humans and animals are more resistant to antibiotics [10].

2. Materials and Methods

2.1. Samples Collection and Identification of Aeromonas hydrophila

A total of (294) diarrheic stool samples were collected between December (2012) and February (2013) from patient children their ages were between (1month_6years) and suffering from diarrhea disease. These stool samples were obtained from Al-Muthana public health laboratory. All suspected samples were activated in APW media at (37) ^{0}C for (18-24)h, and growing on culture media which are TCBS, MacCon key and Blood agar at (37) ^{0}C for (18-24)hr [11]. To confirm initial diagnosis of bacteria a manual biochemical tests were used such as catalase, oxidase, indole, methyl red, simmone citrate, vogues-proskauer, lysine decarboxylase, Kligler iron test, gelatin liquefaction, and string test [12]. To identify A.hydrophila by amplify genes of (16) Sr RNA gene from genomic DNA a polymerase chain reaction (PCR) technique was used.So according to the genomic DNA purification kit supplemented by the manufacturing company (Geneaid/Taiwan) the DNA extraction from Gram negative bacteria was performed. Both Gel electrophoresis and UV transillluminator have been used for detection of DNA [13]. According to the [11.14] recommendations the primers selection were used for diagnosis A.hydrophila, and it as shown in Table (1) the primers synthesized by AccuOligo- Bioner Company, Korea,

Table (1): The sequence of forward and reverse primers

Primer type	Primer sequence	Product size
Forward *16Sr RNA* - F	5-CCAGCAGCCGCGGTAATACG-3	300 bp
Reverse *16Sr RNA* - R	5-TACCAGGGTATCTAATCC-3	

Abstract

A total of (294) stool samples were collected from patient children, their ages were between 1 month to (6) years and suffering from diarrhea disease during period December (2012) and February (2013) from Al-Muthana public health laboratory in Muthana province - Iraq. All suspected isolates were screening by traditionally tests and then confirmed by PCR technique (16S r RNA gene).The results showed that; there were (12) positive isolates of Aeromonas hydrophila. Then investigation was done on some virulence factors and all isolates were showed its ability to produce haemolysin, protease, lipase, phospholipase, capsule and motility. As well as, the antibiotic susceptibility of these isolates were taking in the account and showed that all the isolates were multiple antibiotic resistance to the Amoxicillin, Ampicilin, Cephalothin and Cefotaxime. While, all isolates were sensitive to the Nalidixic acid, Tetracycline and Gentamicin.

Keywords

A. hydrophila isolation, investigate of some virulence factors and antibiotic susceptibility.