



Spectroscopic, potentiometric and Antimicrobial Activity of Ni(II) and Cu(II) Complexes with Schiff base Derived from salicylaldehyde and 2-aminophenol

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ABSTRACT

The Schiff base chelate of Cu(II) and Ni(II) derived from condensation reaction of salicylaldehyde and 2-aminophenol have been synthesized and investigated by different physicochemical techniques. The resulting complexes were characterized by elemental analysis, molar conductance, FT-IR, melting point or decomposition measurements, solubility in different solvent. The elemental analysis data show the formation of 1:1 metal to ligand ratio. The Schiff base act as a tridentate ligand, coordinated through deprotonated phenolic oxygen and azomethine Nitrogen atom. The synthesized chelates were assayed for antibacterial activity against some pathogenic bacteria: *Staphylococcus aureus*, and *Escherichia coli*, and the fungi: *Aspergillus niger* and *Candida albicans*, using disc diffusion method. The synthesized Schiff base complexes exhibit higher antimicrobial activity than the free Schiff base due to the chelation.

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Introduction

Schiff base compounds have been widely studied and used attracting much attention to both organic synthesis and metal ion complexation reaction. Schiff bases are obtained from condensation reaction of either an aldehyde or ketone with an amine to form stable compound containing of $-C=N$ group called Imine (Pierre, 1987). The Schiff bases are characterized by the resultant imine functional group and for binding metals ion via the N-atom lone pair especially in combination with one or more donor atoms to form polydentate or chelating ligand. The importance of Schiff base is due to its versatile nature and ability to react with metals generally. Schiff bases and their transition metals complexes have been known, the complexes are formed by the interaction between metal ions and ligands (Schriver et al., 1994). This is done by the donation of one or more lone pair(s) of electrons in the ligand to the metal empty d-orbital of suitable energy. The coordination results in the formation of neutral cationic and anionic complexes (Hridia et al., 1995). Tsumaki, (1983) reported $[Co(sal_2-en)]$ Complex which received a great attention following to its ability to undergo reversible adduct formation with molecular oxygen. The oxygenation ability of the complex was first recognized by Hassan et al., (1998), however, the mechanism for the oxygenation process was not well understood until recently with the advent of modern physical techniques. In another report, Xishiet al. (2003) described the synthesis and spectroscopic properties of manganese(II), cobalt (II) and copper (II) complexes with novel Schiff base ligand derived from 2, 2' bis(p-methoxyphenylamine) and salicylic aldehyde. Transition metal Schiff base complexes are used in various fields, such as medicine, agriculture, industries etc. Example, $[Co(acac_2-en)]$ in dimethylformamide, pyridine and substituted pyridines proved to be involved in oxygen metabolism (Hanna and Mona, 2001). The family of Schiff bases is important intermediates for the synthesis of some bioactive compounds such as β -lactam (Thomas, 2007). Furthermore, they are reported to show a

variety of interesting biological actions including antibacterial (Jarrahpouret al., 2004; Baluja et al., 2006) and antifungal (Dhakery and Saxena, 2007; Dash et al., 1987; Patel and Jejurka, 1994) and catalysts (Nashinaga et al., 1998). Oxovanadium Complexes have been found strongly active, against some type of Leukemia (Dong et al, 2002). Transition metal complexes derived from a number of amino acids have been reported to have biological activity (Zahid et al, 2007). A method for controlling bacterial and fungal diseases in plants by applying chelate complexes is used to control fungal infection which causes pre-harvest damage to crops by killing them so as to decrease yield and render the plants susceptible to other infection. Recent advance in research in Schiff base and their transition metals complexes has shown that, Vanadium containing compounds have utility as insulin is also suggested that Vanadium compounds have acted as anti-tumor agent. This stimulated their design and synthesis for biological significance and natural process transfer (Tuovinen et al., 1988).

This paper reports synthesis, spectroscopic, potentiometric and biological activity of nickel (II) and copper (II) complexes with Schiff base derived from Salicylaldehyde and 2-aminophenol

Experimental

All chemicals used in this work were reagent grade obtained from Sigma Aldrich, United Kingdom and used without further purification. All weighing were observed on electrical mettle balance model AB54, pH measurement were performed using a Jenway pH meter model 3320. Conductivity was measured using Jenway conductivity meter model 4010. Infra-red spectral analysis was recorded using Perkin Elmer spectrum 100 Fourier transformer IR spectrometer model within the range of 500-400 cm^{-1} . The invitro antimicrobial activity was performed using disc diffusion method, the two pathogenic bacteria viz *Escherichia coli* and *staphylococcus aureus* and two fungi *Aspergillus niger* and *Candida albicans* were obtained from

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Preparation of Schiff base

Schiff base was prepared by reacting 1.7g (0.014mol) of Salicylaldehyde in 20cm³ absolute ethanol and 1.5g of O-aminophenol in 30cm³ absolute ethanol. The mixture was concentrated by heating to 25cm³ and then cooled in an ice-bath. An orange crystalline product was formed, washed with hot ethanol before recrystallizing from ethanol and drying in vacuum desiccators over phosphorus pentoxide for two days (Islam *et al.*, 2001).

Preparation of Copper (II) and Nickel (II) Complexes

A 25cm³ of ethanolic solution of Cu(II) or Ni(II) chloride was added to 30cm³ of ethanolic solution of the prepared Schiff base. The resulting mixture was boiled on a water bath for 5 min and cooled. The complex was separated, washed with hot ethanol and dried in vacuo over P₄O₁₀ for two days. (Islam *et al.*, 2001)

Determination of dissociation constant of Schiff base

To a 400cm³ beaker, 90cm³ of distilled water was added followed by 100cm³ of 0.2mol dm⁻³ KNO₃, 10cm³ of 0.4mol dm⁻³ solution of the Schiff base and the solution of NaOH (0.48mol dm⁻³) was added gradually and the corresponding pH value recorded after each addition. The dissociation constant *pK_a* of the Schiff base was calculated using equation (Gregory *et al.*, 1978)

Determination of dissociation constant of the complexes

Into a 40cm³ beaker 100cm³ of 0.2m KNO₃, 1mmole of Cu(II) or Ni(II) chloride, 0.1m HNO₃ and 90cm³ of distilled water were added, respectively. A magnetic stirring bar and sodium salt of the Schiff base prepared by neutralizing a known quantity of the Schiff base with calculated amount of standardized NaOH solution. After each 0.2cm³ aliquot addition the corresponding pH of the stirred reaction mixture was recorded. From the result obtained, stability constant of the complex compound and the number of the coordinate ligands per metal ion were determined (Gregory, *et al.*, 1978)

Antibacterial and Antifungal activity

The paper discs were impregnated with 1000, 2000 and 3000µg concentration of the Schiff base and its metal (II) complexes. Two loopfuls of the standard inoculum were evenly streaked on the plates in duplicates. Discs containing the impregnated quantities of the complexes as well as the control discs (with only DMSO) were placed firmly on the surface of the medium by means of sterile syringe needle at 40mm apart. For the bacteria the plates were incubated at 37°C for one day (shamsuddeen *et al.*, 2008). For the fungal activity, it was incubated at room temperature for two days (Hassan, *et al.*, 2006). Each of the plates was examine for clear zone of inhibitions. The diameters of the zone of inhibitions were measured with millimeter rule and the mean recorded in the nearest millimeter.

Result and discussion

The Schiff base ligand was prepared as orange crystalline solid. The percentage yield recorded was 81.38 as shown in Table 1. Solubility test (Table 2) carried out on the ligand and its Ni(II) and Cu(II) complexes in some common solvents showed that, it is soluble in ethanol, methanol and DMSO but insoluble in water, Benzene and Ether, while in Toluene is slightly soluble. The molar conductance of synthesized complexes (Table 3) was measured using 10⁻³M DMSO solvent at room temperature. The conductivity values are in the range of 3.00-5.00 Ohm⁻¹ cm² mol⁻¹. These values are too low to account for any dissociation of the complexes in DMSO and the obtained

value were taken as a good evidence of the existence of a non-electrolytic in nature of the complexes (Geary, 1972). The important IR frequencies exhibited by the Schiff base ligand and their complexes are tabulated in Table 4, the ligand shows broad band at 3510cm⁻¹ due to the phenolic hydroxyl group, which show shift in frequency in complexes, indicating the coordination through metal atom (Synmalet *et al.*, 1984) the band in the 1600cm⁻¹ are observed due to $\nu(\text{C}=\text{N})$ which has been shifted toward lower region at around 1550-1565cm⁻¹ in the complexes indicating the participation of the azomethine group in the complexes formation (Makodeet *et al.*, 2004). The sharp band at 3540 and 3530cm⁻¹ for Ni(II) and Cu(II) Schiff based complex are due to $\nu(\text{O}-\text{H})$ stretching vibration indicating the presence of coordinated water (Byeong-Goo *et al.*, 1996). The appearance of the two new band in the region 749-750 and 596-610 cm⁻¹ in the metal chelates suggests the formation of M-O and M-N bonds respectively (Saleenet *et al.*, 2003). The dissociation constant of the Schiff base determined (Table 8) is 10.27 indicating that it is a weak acid (Gregory 1978). The potentiometric analytical result of Ni(II) and Cu(II) Schiff base complex established 1:1 metal -Schiff base ratio (Table 9 and 10), while the stability constants of the Ni(II) and Cu(II) complexes determine are 5.7544×10^6 and 3.4673×10^7 and the corresponding change in Gibb's free energy are -3.8546×10^{-1} and -4.2994×10^{-1} KJmol⁻¹ respectively (Table 5). The antibacterial activity tested for the Schiff base and the metal (II) complexes (Table 6) have been determined. The diameter of inhibition zone (mm) was measured for each treatment. The Schiff base ligand has less activity in comparison with their complexes against *Escherichia coli* and *Staphylococcus aureus* this is due to the fact that the metal chelates have more antibacterial activity than uncomplexed ligand because of the chelation theory (Mandalet *et al.*, 2002). Sensitivity of fungal isolate (*Aspergillusniger* and *Candida albican*) show that the Schiff base and complexes is more active against *Aspergillusniger* than *Candida albican* especially at higher concentration (Table 7). The result for both antibacterial and antifungal studies shows that the complexes show more activity than the ligand under similar experimental conditions. This would suggest that chelation considerably reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with the donor groups and possible electron delocalization over the whole chelate ring. Such chelation could also enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane (suto *et al.*, 1997).

From the result of the analyses carried out the general formula below is suggested.

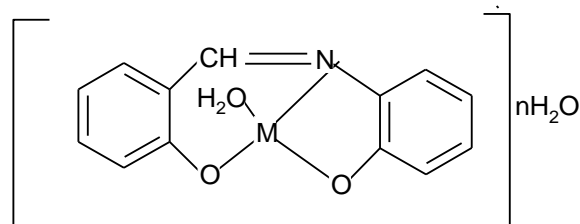


Figure 1: Proposed structure of the complex

Where M is Ni(II) or Cu(II). And n is the number of coordinated water molecules.

Reference

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Table 1: Physical properties of Schiff base and its complexes

Compound	Colour	M.P(^o C)	Decomposition Temp(^o C)	% Yield
Schiff base	Orange	96	-	81.38
[Ni(L)]. 2H ₂ O	Pale yellow	-	110	58.10
[Cu(L)]. 2H ₂ O	Black	-	136	68.54

Table 2: Solubility of Schiff base and its complexes

Compound	Distilled water	Benzene	Toluene	Ethanol	DMSO	Ether	Methanol
Schiff base	IS	IS	SS	S	S	IS	S
[Ni(L)]. 2H ₂ O	IS	IS	SS	S	S	IS	S
[Cu(L)]. 2H ₂ O	IS	IS	SS	S	S	IS	S

KEY: S= Soluble, SS= Slightly Soluble, IS= Insoluble.

Table 3: Molar conductance measurement

Compound	Electrical conductance Ohm ⁻¹	Molar conductance (Ohm ⁻¹ cm ² mol ⁻¹)
[Ni(L)]. 2H ₂ O	3.55 x 10 ⁻⁶	3.6
[Cu(L)]. 2H ₂ O	4.76 x 10 ⁻⁶	4.8

Table 4: Infrared spectral data of ligand and its complexes

Compound	$\nu(\text{O-H})(\text{cm}^{-1})$	$\nu(\text{C=N})(\text{cm}^{-1})$	$\nu(\text{M-O})(\text{cm}^{-1})$	$\nu(\text{M-N})(\text{cm}^{-1})$
Schiff base	3510	1600	-	-
[Ni(L)]. 2H ₂ O	3540	1550	749	596
[Cu(L)]. 2H ₂ O	3530	1565	750	610

Table 5: Stability constant and Gibb's free energy

Compound	Stability constant	Gibb's free energy
[Ni(L)]. 2H ₂ O	5.7544 x 10 ⁶	-3.8546 10 ⁻¹ KJmol ⁻¹
[Cu(L)]. 2H ₂ O	3.4673 x 10 ⁷	-4.2994 10 ⁻¹ KJmol ⁻¹

Table 6: Antibacterial effect of ligand and its complexes

Compound	<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>		
	1000µg/ml (mm)	2000µg/ml (mm)	3000µg/ml (mm)	1000µg/ml (mm)	2000µg/ml (mm)	3000µg/ml (mm)
Schiff base	-	-	5	5	10	12
[Ni (L)]. 2H ₂ O	9	13	15	-	-	-
[Cu (L)]. 2H ₂ O	10	14	15	9	11	16

Table 7: Antifungal effect of ligand and its complexes

Compound	<i>Aspergillusniger</i>			<i>Candida albican</i>		
	1000µg/ml (mm)	2000µg/ml (mm)	3000µg/ml (mm)	1000µg/ml (mm)	2000µg/ml (mm)	3000µg/ml (mm)
Schiff base	6	9	9	-	10	10
[Ni (L)]. 2H ₂ O	7	11	17	-	-	17
[Cu (L)]. 2H ₂ O	-	-	12	-	-	-

Table 8: Dissociation Constant (pKa) of the Schiff Base

S/N	Volume of NaOH (cm ³)	Ph	[H ⁺]	[OH ⁻]	[Na ⁺]	A _{tot}	pKa
1.	0.5	9.85	9.4515x10 ⁻¹¹	7.7625x10 ⁻⁵	1.1970x10 ⁻³	1.9950x10 ⁻²	11.2504
2.	1.0	9.87	9.0261x10 ⁻¹¹	8.1283x10 ⁻⁵	2.3881x10 ⁻³	1.9900x10 ⁻²	10.9268
3.	1.5	9.91	8.2319x10 ⁻¹¹	8.9125x10 ⁻⁵	3.5732x10 ⁻³	1.9851x10 ⁻²	10.7564
4.	2.0	9.98	7.0065x10 ⁻¹¹	1.0471x10 ⁻⁴	4.7525x10 ⁻³	1.9802x10 ⁻²	10.6678
5.	2.5	10.05	5.9635x10 ⁻¹¹	1.2303x10 ⁻⁴	5.9259x10 ⁻³	1.9753x10 ⁻²	10.6054
6.	3.0	10.11	5.1940x10 ⁻¹¹	1.4125x10 ⁻⁴	7.0936x10 ⁻³	1.9704x10 ⁻²	10.5476
7.	3.5	10.14	4.8473x10 ⁻¹¹	1.5136x10 ⁻⁴	8.2555x10 ⁻³	1.9656x10 ⁻²	10.4684
8.	4.0	10.19	4.3202x10 ⁻¹¹	1.6982x10 ⁻⁴	9.4118x10 ⁻³	1.9608x10 ⁻²	10.4143
9.	4.5	10.26	3.6771x10 ⁻¹¹	1.9953x10 ⁻⁴	1.1707x10 ⁻²	1.9512x10 ⁻²	10.4143
10.	5.0	10.31	3.2772x10 ⁻¹¹	2.2307x10 ⁻⁴	1.1707x10 ⁻²	1.9512x10 ⁻²	10.3322
11.	5.5	10.38	2.7893x10 ⁻¹¹	2.6303x10 ⁻⁴	1.2847x10 ⁻²	1.9512x10 ⁻²	10.2265
12.	6.0	10.43	2.4860x10 ⁻¹¹	2.9512x10 ⁻⁴	11.3981x10 ⁻²	1.9417x10 ⁻²	10.2265
13.	6.5	10.47	2.2673x10 ⁻¹¹	3.2359x10 ⁻⁴	1.5109x10 ⁻²	1.9370x10 ⁻²	10.1360
14.	7.0	10.51	2.0678x10 ⁻¹¹	3.5481x10 ⁻⁴	1.6232x10 ⁻²	1.9324x10 ⁻²	10.0211
15.	7.5	10.54	1.9297x10 ⁻¹¹	3.8019x10 ⁻⁴	1.7349x10 ⁻²	1.9277x10 ⁻²	9.8481
16.	8.0	10.56	1.8429x10 ⁻¹¹	3.9811x10 ⁻⁴	1.8462x10 ⁻²	1.9231x10 ⁻²	9.5448
17.	8.5	10.59	1.7199x10 ⁻¹¹	4.2658x10 ⁻⁴	1.9568x10 ⁻²	1.9185x10 ⁻²	8.1218

Average Dissociation Constant (pKa) = 10.27

Table 9: Estimation of number of coordinated ligands in Nickel (II) Complex

S/N	Volume of sodium ligamate (cm ³)	P ^H	[H ⁺]	[OH ⁻]	Log [A]	M _{TOTAL}	N
1.	1.4	3.03	6.2445 x 10 ⁻⁴	1.1588 x 10 ⁻¹¹	-3.6374	0.0049603	0.5643
2.	1.6	3.08	5.5654 x 10 ⁻⁴	1.3001 x 10 ⁻¹¹	-3.6595	0.0049504	0.5786
3.	1.8	3.13	4.9602 x 10 ⁻⁴	1.4588 x 10 ⁻¹¹	-3.6651	0.0049505	0.6981
4.	2.0	3.19	4.3202 x 10 ⁻⁴	1.6749 x 10 ⁻¹¹	-3.6570	0.0049456	0.7651
5.	2.2	3.21	4.1257 x 10 ⁻⁴	1.7539 x 10 ⁻¹¹	-3.6516	0.0049407	0.8411
6.	2.4	3.26	3.6771 x 10 ⁻⁴	1.9679 x 10 ⁻¹¹	-3.6336	0.0049358	0.9119
7.	2.6	3.32	3.2026 x 10 ⁻⁴	2.2594 x 10 ⁻¹¹	-3.36050	0.0049261	0.9367
8.	2.8	3.36	2.9208 x 10 ⁻⁴	2.4774 x 10 ⁻¹¹	-3.5827	0.0049213	1.0562
9.	3.0	3.42	2.5439 x 10 ⁻⁴	2.8445 x 10 ⁻¹¹	-3.5452	0.0049213	1.0985
10.	3.2	3.48	2.2156 x 10 ⁻⁴	3.2659 x 10 ⁻¹¹	-3.5039	0.0049164	1.1654
11.	3.4	3.52	2.0678 x 10 ⁻⁴	3.4995 x 10 ⁻¹¹	-3.4821	0.0049116	1.1895
12.	3.6	3.57	1.9009 x 10 ⁻⁴	4.0179 x 10 ⁻¹¹	-3.4365	0.0049068	1.2065
13.	3.8	3.63	1.5686 x 10 ⁻⁴	4.6132 x 10 ⁻¹¹	-3.3886	0.004902	1.2750
14.	4.0	3.88	8.1283X10 ⁻⁵	8.1283X10 ⁻¹¹	-3.1725	0.0048972	1.3116
15.	4.2	4.02	6.3899X10 ⁻⁵	1.220X10 ⁻¹⁰	-3.0440	0.0048924	1.3733
16.	4.6	4.10	5.3149X10 ⁻⁵	1.3489X10 ⁻¹⁰	-2.9689	0.0048876	1.3884
17.	4.8	4.04	6.1024X10 ⁻⁵	1.1749X10 ⁻¹⁰	-3.0254	0.0048828	1.4078
18.	5.0	4.27	3.5934X10 ⁻⁵	1.9953X10 ⁻¹⁰	-2.8068	0.004878	1.4631
19.	5.2	4.29	3.4356X10 ⁻⁵	2.0893X10 ⁻¹⁰	-2.8068	0.0048733	1.4974
20.	5.4	4.32	3.2026X10 ⁻⁵	2.2387X10 ⁻¹⁰	-2.7880	0.0048685	1.5153
21.	5.6	4.34	3.0584X10 ⁻⁵	2.3442X10 ⁻¹⁰	-2.7586	0.0048638	1.5829
22.	5.8	4.35	2.9888X10 ⁻⁵	2.3988X10 ⁻¹⁰	-2.7392	0.0048591	1.5973
23.	6.0	4.38	2.7893X10 ⁻⁵	2.5704X10 ⁻¹⁰	-2.70041	0.0048544	1.6422
24.	6.2	4.39	2.7258X10 ⁻⁵	2.6303X10 ⁻¹⁰	-2.7295	0.004897	1.6881
25.	6.4	4.41	2.6032X10 ⁻⁵	2.7542X10 ⁻¹⁰	-2.6906	0.004845	1.7263
26.	6.6	4.44	2.4294X10 ⁻⁵	2.9512X10 ⁻¹⁰	-2.6713	0.0048403	1.8654
27.	6.8	4.46	2.3201X10 ⁻⁵	3.1623X10 ⁻¹⁰	-2.6420	0.0048356	1.9356
28.	7.0	4.47	2.2673X10 ⁻⁵	3.1623X10 ⁻¹⁰	-2.6225	0.0048309	2.0316
29.	7.2	4.48	2.2156X10 ⁻⁵	3.2659X10 ⁻¹⁰	-2.6128	0.004863	2.3582
30.	7.4	4.49	2.1652X10 ⁻⁵	3.3113X10 ⁻¹⁰	-2.6029	0.0048216	2.4609

Average n value = 1.3711

Table 10: Estimation of number of coordinated ligands in Copper (II) Complex

S/N	Volume of sodium ligamate (cm ³)	P ^H	[H ⁺]	[OH ⁻]	Log [A]	M _{TOTAL}	N
1.	1.2	2.69	1.3662 x 10 ⁻³	5.2966 x 10 ⁻¹¹	-3.9664	0.0049652	0.6339
2.	1.4	2.71	1.3047 x 10 ⁻³	5.5463 x 10 ⁻¹²	-3.8665	0.0049603	0.6543
3.	1.6	2.76	1.1628 x 10 ⁻³	6.2230 x 10 ⁻¹²	-3.5444	0.0049554	0.6934
4.	1.8	2.82	1.0127 x 10 ⁻³	7.1449 x 10 ⁻¹²	-3.3765	0.0049505	0.7356
5.	2.0	2.88	8.8206 x 10 ⁻³	8.2035 x 10 ⁻¹²	-3.2844	0.0049456	0.7589
6.	2.2	2.93	7.8614 x 10 ⁻⁴	9.2045 x 10 ⁻¹²	-3.4928	0.0049407	0.7887
7.	2.4	2.96	7.3367 x 10 ⁻⁴	9.8628 x 10 ⁻¹²	-3.5581	0.0049358	0.8263
8.	2.6	2.99	6.8470 x 10 ⁻⁴	1.0568 x 10 ⁻¹¹	-3.6014	0.004931	0.8649
9.	2.8	3.05	5.9634 x 10 ⁻⁴	1.2133 x 10 ⁻¹¹	-3.6487	0.0049261	0.8981
10.	3.0	3.07	5.6950 x 10 ⁻⁴	1.2705 x 10 ⁻¹¹	-3.6487	0.0049261	0.9351
11.	3.2	3.09	5.4388 x 10 ⁻⁴	1.2705 x 10 ⁻¹¹	-6.587	0.0049213	0.9775
12.	3.4	3.11	5.1939 x 10 ⁻⁴	1.3305 x 10 ⁻¹¹	-3.6618	0.0049116	1.0648
13.	3.6	3.12	5.0757 x 10 ⁻⁴	1.4256 x 10 ⁻¹¹	-6.650	0.0049068	1.2546
14.	3.8	3.15	4.7369 x 10 ⁻⁴	1.5276 x 10 ⁻¹¹	-3.6639	0.004902	1.2740
15.	4.0	3.17	4.5238X10 ⁻⁴	1.5849X10 ⁻¹¹	-3.6612	0.0048972	1.2903
16.	4.2	3.18	4.4208X10 ⁻⁴	1.6218X10 ⁻¹¹	-3.6593	0.0043924	1.3164
17.	4.6	3.22	4.0318X10 ⁻⁴	1.7783X10 ⁻¹¹	-3.6485	0.0048876	1.3765
18.	4.8	3.23	3.9400X10 ⁻⁴	1.8197X10 ⁻¹¹	-3.6451	0.0048828	1.4174
19.	5.0	3.24	3.8503X10 ⁻⁴	1.8620X10 ⁻¹¹	-3.6415	0.004878	1.4673
20.	5.2	3.25	3.7627X10 ⁻⁴	1.9055X10 ⁻¹¹	-3.6377	0.0048733	1.5386
21.	5.4	3.26	3.6771X10 ⁻⁴	1.1949X10 ⁻¹¹	-3.6336	0.0048685	1.6268
22.	5.6	3.27	3.4445X10 ⁻⁴	1.9953X10 ⁻¹¹	-3.6248	0.0048638	1.6972
23.	5.8	3.28	3.5115X10 ⁻⁴	2.0417X10 ⁻¹¹	-3.6202	0.0048591	1.7086
24.	6.0	3.29	3.4316X10 ⁻⁴	2.1086X10 ⁻¹¹	-3.6153	0.0048544	1.8122
25.	6.2	3.30	3.3535X10 ⁻⁴	2.1379X10 ⁻¹¹	-3.6050	0.0048497	1.8759
26.	6.4	3.32	3.2026X10 ⁻⁴	2.2387X10 ⁻¹¹	-3.5827	0.004845	1.9247
27.	6.6	3.36	2.9208X10 ⁻⁴	2.4547X10 ⁻¹¹	-3.5582	0.0048403	1.9788
28.	6.8	3.40	2.6638X10 ⁻⁴	2.6915X10 ⁻¹¹	-3.5110	0.0048356	2.1530
29.	7.0	3.47	2.2672X10 ⁻⁴	3.1623X10 ⁻¹¹	-3.3805	0.0048309	2.2751
30.	7.2	3.64	1.5329X10 ⁻⁴	4.6774X10 ⁻¹¹	-3.2263	0.0048263	2.4623

Average n value = 1.3427

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