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Derivatization of artemisinin derivatives using 4-carboxyl -2, 6-dinitro benzenediazonium (CDNBD) ion

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Abstract

A new UV-spectrophotometric method for analysis of artesunate, dihydroartemisinin and artemether has been developed. The reaction involves the diazo coupling of acid decomposition product generated *in situ* with the highly reactive 4- carboxyl-2,6- dinitrobenzene diazonium ion (CDNBD) to form azo adducts that are UV active. Optimum reaction conditions were established at 70oC for 10 min (artesunate and artemether) and 5 min (dihydroartemisinin). The diazotized compounds were determined at λ max of 320 nm (artesunate and dihydroartemisinin) and 310 nm (artemether). Assays were linear over 3.6-28.8 (artesunate), 5.2-26.0 (dihydroartemisinin) and 5.4-21.6 µg/mL (artemether) with LODs of 0.95, 0.17 and 0.55 µg/mL respectively. Overall recovery of 99.45 ± 2.75 (artesunate), 99.88 ± 2.41 (dihydroartemisinin) and 99.97 ± 1.53% (artemether) were obtained from the three-day assessment of accuracy and repeatability.

The procedure was successfully applied to the determination of artemisinin derivatives in tablets and injectables. There was no significant difference ($p>0.05$) with official methods. The new method has the advantages of speed, cost-effectiveness and simplicity.

Keywords: Artesunate, Dihydroartemisinin, Artemether, CDNBD, Diazo coupling reaction.

Introduction

Artemisinin is the antimalarial principle isolated by Chinese scientists from Artemisia annua L. Artemisinin is a drug used to treat multi- drug resistant strains of *Plasmodium falciparum* malaria (WHO 2005). Artemisinin (ginghaosu) is a sesquiterpene lactone with a peroxide bridge. It is poorly soluble in oils or water but the parent compound has yielded dihydroartemisinin, the oil soluble derivatives artemether and artether and the more water soluble derivatives, sodium artesunate and artelinic acid. These derivatives have more potent blood schizonticidal activity than the parent compound and are the most rapidly effective antimalarial drugs known. They are used for the treatment of severe and uncomplicated malaria (McIntosh and Olliaro 1998).

The artemisinin derivatives do not have any significant light absorption in the workable wavelength region of the UV-VIS spectroscopy and they do not have particular chemical groups that easily react with certain reagents to yield coloured products; however, they can be transformed by acid or base treatment to more reactive compounds such as enolate/carboxylates or α,β-unsaturated decalones (Zhao and Zeng 1986, Thomas et al 1992). This transformation has been used as the basis for the determination

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of these drugs in dosage forms and biological fluids (Zhao 1987, Idowu et al. 1989, van Agtmael 1998) using HPLC. The reactive methylene centres generated by acid or base treatment has also been used for the colorimetric detection of counterfeit artesunate, dihydroartemisinin (Green et al. 2000) and artemether (Green et al. 2001). Some other previously described methods for the assay of artemisinins are thin layer chromatography using spray reagents such as acidified 4-methoxybenzaldehyde reagent in methanol-water (Gabriels et al. 2004) or *p*-dimethylamino benzaldehyde and heated at 80°C; HPLC without prior derivatization using electrochemical detectors (Acton et al. 1985, Thomas et al. 1992) or with mass spectrometric detection (Ortelli et al. 2000, Christen and Veuthey 2001, Naik et al. 2005, Liu et al. 2007). However, majority of the HPLC techniques involved precolumn derivatization (Zhao and Zeng 1986, Zhao 1987, Zhou et al. 1987) or post-column derivatization (ElSohly et al. 1987, Liu et al. 2008). Some other methods include capillary electrophoresis (D'Hulst et al. 1996) and bioassay techniques (Jaziri 1993, Ferreira and Janick 1996) and recently HPTLC (Agarwal et al. 2009). Majority of these techniques previously reported suffers from the disadvantage of using high acid concentration and carrying out the reaction at high temperatures for a prolonged period of time. Zollinger (1991) observed that the reactive methylene centres generated by the acid or base decomposition can readily react with diazonium salts and this procedure has been utilized by Green (2000) for the alkali-decomposition product of artesunate with the diazonium salt fast red TR. The avidity with which diazonium ions react with active methylene centres depend on the reactivity of the diazonium ion. In our previous reports, we have demonstrated the high reactivity of the diazonium; 4-carboxyl-2,6-dinitrobenzene diazonium (CDNBD) ion as a highly reactive diazo coupling reagent for secondary amino derivatives (Idowu et al. 2002, Idowu et al. 2006) and phenol ethers (Idowu et al. 2004, Adegoke et al. 2006a, 2006b, 2007a and 2007b). Majority of the previously described methods especially HPLC techniques are difficult to adopt in poor-resource economies. Thus the present method sought to develop simple and accurate procedures for these important antimalarials using the diazonium ion, CDNBD.

Materials and Methods

Chemicals and Reagents

Artesunate (ART), dihydroartemisinin (DHA) and artemether (ATM) chemical reference substances were obtained as gift from Swiss Pharma Nig Ltd, Lagos, Nigeria. Methanol, ethylacetate, hydrochloric acid, sulphuric acid, orthophosphoric acid, sodium hydroxide pellets, phenolphthalein, ethanol (All Analar reagent grade obtained from BDH Chemical Ltd, Poole, England), Glacial acetic acid and iodine crystals were obtained from Sigma Chemical Company, USA. Various dosage forms of the artemisinin derivatives were obtained from reputable Pharmacies in Ibadan, Nigeria.

Equipment

UV-VIS spectrophotometer 2802 (Unico, Shanghai, China), analytical balance (Mettler H80, UK), ultrasonic bath (Langford, UK), vortex mixer (Griffins&George ltd, UK), Equitron water bath (Medica Instrument Mfg Co, Mumbai, India), Cecil HPLC with variable wavelength detector (Cecil Instruments, Cambridge, England).

Preparation of CDNBD solution and reference drug stock solutions

The stock solution of CDNBD (0.918 mmol/L) in sulphuric/orthophosphoric acid mixture was prepared as previously reported (Idowu 2005).

Equimolar concentrations of ART (0.0036 g), DHA (0.0026 g) and ATM (0.0027 g) were prepared by dissolving in 10 mL of glacial acetic acid.

Spot tests

A 0.1 mL quantity of each stock of ART, DHA and ATM were added into different test tubes containing 0.5 mL of CDNBD solution. The colours formed immediately and after 20 min were noted. A different set of sample preparations was made and incubated at 80°C for 5 and 20 min. The colours produced were also observed.

Thin layer chromatographic analysis was carried out on silica gel GF_{254} using the reference samples, CDNBD and the reaction mixture from each reaction test tubes. Development was done in mobile phase mixtures of ethylacetate: methanol (9:1), ethylacetate: methanol (8:2) and ethylacetate: hexane (5:5). Reversed phase TLC was also carried out using reversed-phase TLC plates and methanol: water (4:6) as mobile phase. Identification of spots was done using the UV lamp and vanillin/sulphuric acid as spray reagent.

Optimization studies

In order to determine suitable analytical wavelengths for the reaction product, two critical response parameters (temperature and time) were optimized using the method of steepest ascent (Karnes and March 1993). Aliquots of the artemisinin stocks (100 µL) were added to the reagent solution (500 µL) in test tubes and the reaction mixtures were mixed in a vortex mixer for 10 s followed by incubation in turn at 30°C, 50°C, 60°C, 70°C and 80°C for 5 and 20 min. The reaction was terminated by addition of ice-cold water (5 mL) to the reaction mixture kept in ice-bath. Each aqueous solution was extracted with ethyl acetate (5 mL) and kept in a vial wrapped with aluminium foil. A blank reagent was prepared in similar manner but replacing the drug stock with glacial acetic acid. The absorption spectrum of the reaction mixture extract was determined against the absorption spectrum of the blank reagent extract, using the UV/Visible spectrophotometer. Optimal difference in absorptivity between the reagent and the reaction mixtures were found at 320 nm for ART and 310 nm for DHA and ATM and therefore were selected as working λ_{max}.

The optimal time was determined by adding 100 µL of each artemisinin stock solutions in turn to 500 µL in eight test tubes. Coupling reaction was carried out by incubation at 70°C for 0, 2, 5, 10, 15, 25 and 30 mins. Ethyl acetate extracts of the reaction mixture were prepared as usual after each reaction time and the absorbance readings recorded at 320 nm (ART) and 310 nm (DHA and ATM). An optimal reaction time was taken as the time corresponding to the maximal absorption of the samples. All determinations were done in duplicates.

Stoichiometric ratio of drug-reagent adduct formation

Equimolar solutions of the reagent and the three drug stock solutions were prepared using the procedures described above. In seven different tubes, 0, 0.25, 0.33, 0.5, 0.67, 0.75 and 1.0 mL of the reagent solution were put respectively. Each tube was then made up to 1.0 mL with drug stock solution (ART, DHA and ATM as appropriate). A series of blank determinations were carried out in which the volume of the drug stock solution was replaced with various volumes of CDNBD solution. The mixture was mixed in a vortex mixer for 10 s and kept at 70°C for 10 mins (ART and ATM) and 5 mins. The extraction was carried out afterwards as usual. The absorbance was measured against the blank at the working wavelength of each adduct. Each determination was carried out in duplicates.

Validation of methods

Calibration lines using standard solutions ranging 0-28.8 (ART), 0-26.0 (DHA) and 0-21.6 µg/mL (ATM) were carried out using the optimal analytical conditions as described above. Linear regression analysis was used to calculate the slope, intercept and the coefficient of determination (r^2) of each calibration line.

The limit of detection (LOD) was calculated according to the current ICH guidelines as 3.3 standard deviation of the blank divided by the slope of the calibration curve. Accuracy and repeatability of the new methods were carried out on three successive days as stipulated by the USP 24 (2000).

Assay of Dosage forms

Weight uniformity tests were carried out on each brand of the dosage form obtained for the artemisinin derivatives. The amount of powdered tablets of ART, DHA and ATM equivalent to 0.0036 g, 0.0026 g and 0.0027 g were weighed and dispersed in 10 mL of glacial acetic acid. The samples were vortex mixed for 20 min and thereafte

filtered. For each brand of ART, 0.2 mL quantity of the filtered solution was coupled with 0.5 mL of the reagent and carried through the procedures described above. Six replicate samples were determined. The procedure was repeated for DHA using 0.3 mL quantity of the filtered samples, while for ATM tablets 0.2 mL of the drug stock was used.

The International Pharmacopoeia procedures for the assay of the dosage forms were adopted as reference procedures *viz*: alkalimetric titration with standard NaOH (ART), UV spectrophotometry (DHA) and reversed phase HPLC for ATM. The assay results obtained from the new method and the official methods were statistically compared using F-ratio test, t-test and ANOVA. A 2-tailed probability values less than or equal to 0.05 (95 % confidence interval) was taken as significant.

Interference studies

Two approaches were adopted for the assessment of the influence of commonly utilized excipients on the new method, spiking reference sample into excipients and into dosage forms. For ART, 0.2 ml quantity of artesunate reference stock solution was spiked into each of the tablet excipients; lactose, starch, magnesium stearate, talc, gelatin and a mixture of the excipients, 0.5 mL CDNBD reagent was added and vortex mixed for 10 seconds. The tubes were placed in water bath at 70°C for 5 min and extracted in 5 mL ethylacetate. The absorbance of each sample was taken at 320nm. Similar quantity of the reference solution was added to dosage form solutions (0.2 mL) and processed as usual. Similar procedures were carried out for DHA and ATM. Recoveries of the drug from the two sample matrices were calculated and compared with recoveries obtained in the assessment of accuracy.

Results and Discussions

Evidence of coupling reaction and selection of analytical wavelength

Diazo coupling reaction between CDNBD and the artemisinins gave instant reddish brown colour indicating the formation of an azo dye. The colours produced by the artemisinin derivatives became intense at elevated temperatures. The ethyl acetate extract of the azo adducts gave an orange-coloured solution. Thin layer chromatographic analysis revealed the formation of new products as distinct from the starting materials. The result of the TLC analysis is presented in Table 1 for both the normal and reversed phase TLC carried out.

Table 1. R_f values for the thin layer chromatographic analysis of reaction mixture

		Normal phase		
Mobile phases	**ART adduct**	**DHA adduct**	**ATM adduct**	**CDNBD**
EtOAc: MeoH (9:1)	0.82 (0.96)	0.84 (0.95)	0.82 (0.91)	0.65
EtOAc: MeOH (8:2)	0.91 (0.99)	0.94 (0.96)	0.93 (0.90)	0.74
EtOAc : Hexane (5:5)	0.77 (0.80)	0.87 (0.91)	0.80 (0.85)	0.72
		Reversed phase		
MeOH : H_2O (4:6)	0.27 (0.17)	0.25 (0.13)	0.27 (0.14)	0.80

R_f values of pure artemisinin derivatives in parentheses

The azo adducts were less lipophilic than the artemisinin derivatives giving lower R_f values in normal phase and higher R_f in reversed phase mode. The overlaid absorption spectra of the ART-, DHA- and ATM-CDNBD azo adducts are presented in Figure 1.

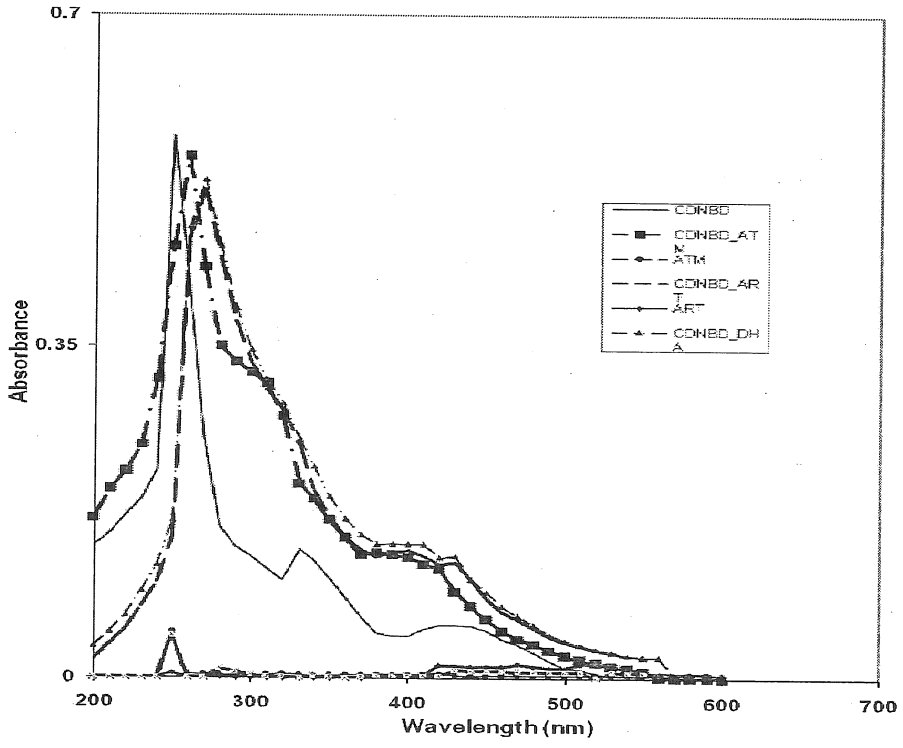
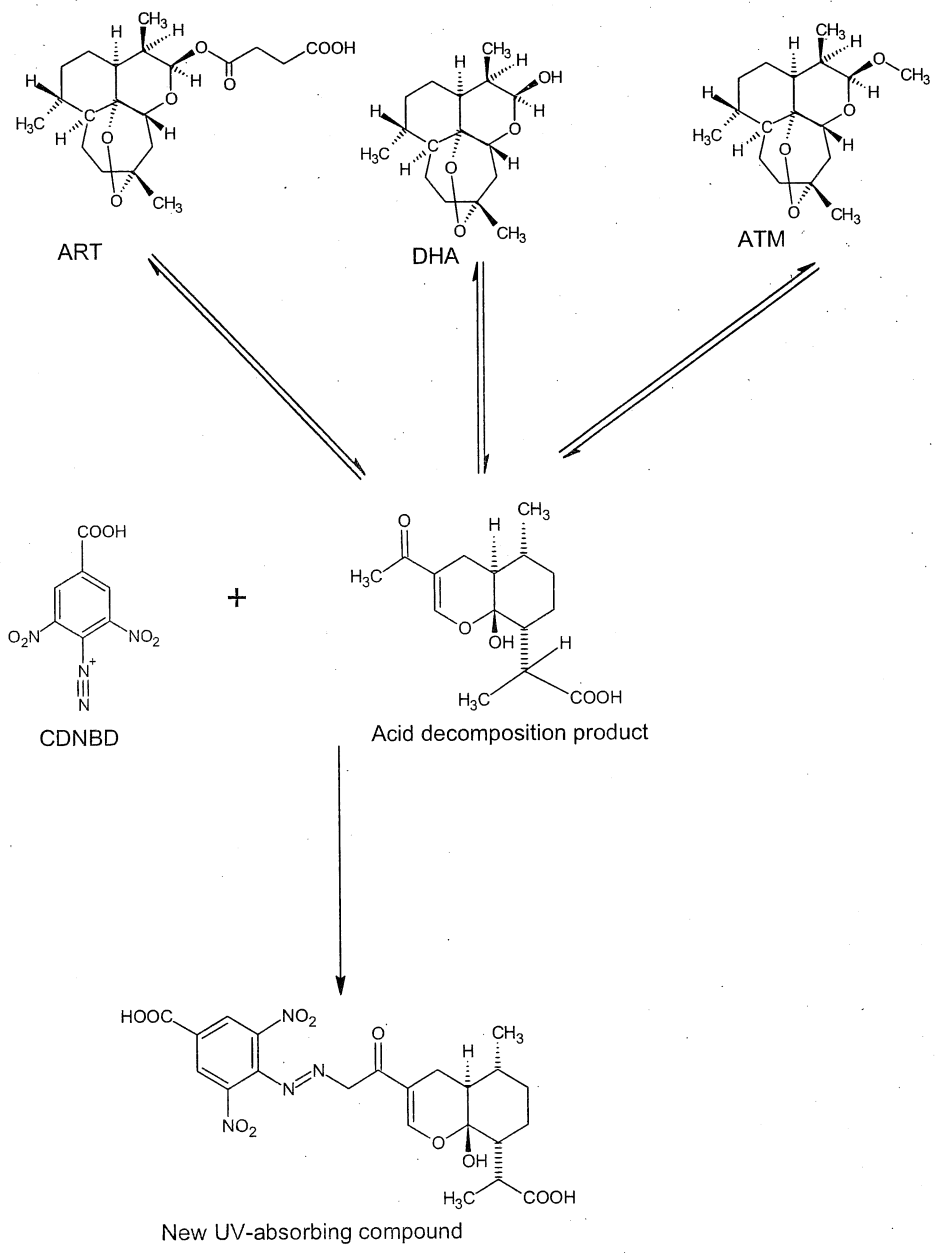


Figure 1. Overlaid absorption spectra of derivatized artemisinin types on CDNBD

When compared to the spectrum of CDNBD, a significant hyperchromic shift in absorption spectra was observed. However, there was also a good bathochromic shift with respect to the reagent. Two clearly defined peaks were observed for the three artemisinin derivatives at the points where CDNBD has defined valleys; 300-320 nm and 380-420 nm and additional slight bathochromic shift in the region 260-270 nm for the three adducts. Optimal difference in absorptivity was found at 320 nm (ART) and 310 nm (DHA and ATM) where CDNBD has its first valley. These wavelengths were therefore selected as the analytical wavelengths. When compared with the near zero absorptivity of the artemisinin derivatives, CDNBD will be a useful derivatizing reagent for these drugs. The preparation of CDNBD in acidic medium offers the advantages that the acid decomposition of the artemisinin derivatives will take place *in situ*. This will preclude the use of prior decomposition before the azo adduct formation will take place. Based on the previous description of the formation of α,β-unsaturated decalones by Thomas et al (1992) following acid decomposition, the azo adduct between the artemisinin derivatives and CDNBD is proposed as shown in Scheme 1. Further evidence for the possibility of the formation of similar azo adduct is derived from the similar pattern of the absorption spectra of the three artemisinin derivatives depicted in Figure 1.



Scheme 1. Coupling reaction pattern for the formation of azo adduct between artemisinins and CDNBD

Optimization studies

Optimization of temperature and time carried out at the five temperature levels reveal the superiority of carrying out the reaction at 70°C over the other temperature conditions. For all the artemisinin derivatives, absorbance values rose gradually from 30 to 70°C and declined at 80°C. However, at 20 min, the corresponding absorbance values for artesunate and artemether were higher than that of 5 min. Decomposition of azo adducts produced is suggestively taking place at 80°C. The results for the optimization of temperature are presented in Figure 2.

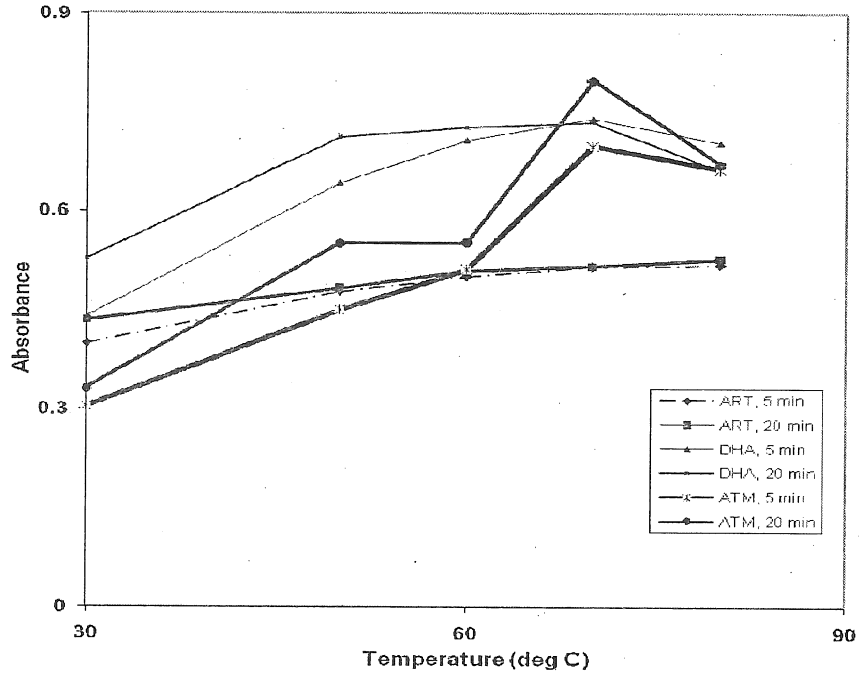


Figure 2. Optimization of temperature for the coupling reaction between artemisinin derivatives and CDNBD

Figure 3 shows the result for the optimization of the time required for optimal detector response at 70°C. For artesunate, the absorbance value rose gradually and peaked at 10 min. It was constant at 15 min and began to drop gradually from 20 min.

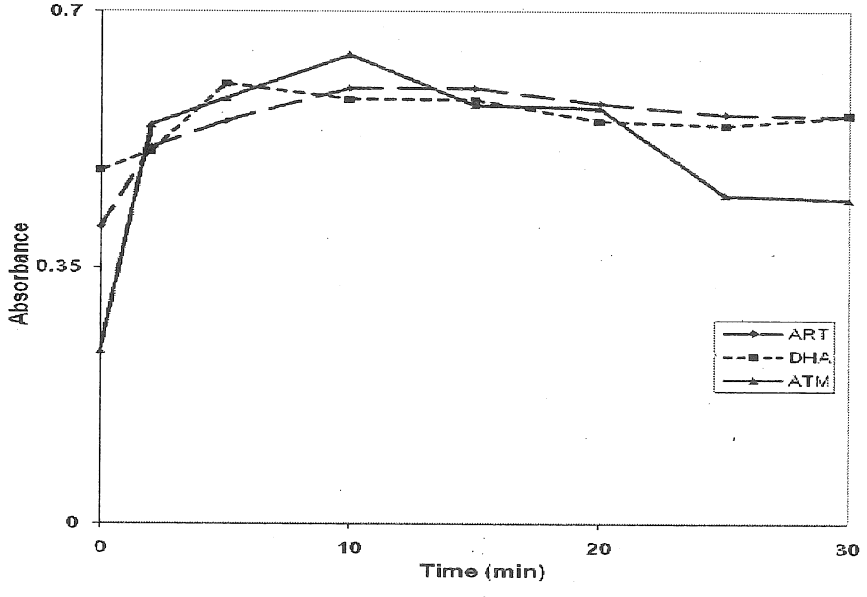


Figure 3. Optimization of coupling reaction time at 70°C

Ten minutes was therefore selected as the optimal time for coupling to take place. Similar experiments for DHA and ATM revealed 5 and 10 mins as optimal times for coupling. Thus the acid medium in

which CDNBD is prepared aided by the elevated temperature provided a means for the spectrophotometric determination of the artemisinin derivatives through azo dye formation. This new method has obvious advantages over the use of fast red described by Green et al (2001); the reaction does not involve the use of buffer systems, CDNBD can last up to 90 days at 0°C (Idowu et al. 2005), the acid decomposition product is produced almost immediately in the acid medium and the distinct orange colour in ethylacetate can be used as an identification procedure.

The stoichiometric ratio was carried out using Job's continuous variation method. Optimal absorbance values were obtained at the following reagent to drug ratios; 3:1 (ART), 1:1 (DHA) and 2:3 (ATM). While the possibility of formation of multiple products may be occurring this was not obvious in the TLC procedures carried out as single spots were obtained in both the normal and reversed phases adopted. It has however been discovered from our previous work that higher diazonium concentration is necessary for azo dye formation (Adegoke 2007b) while spectroscopic characterization revealed the formation of a single compound (Adegoke 2008). It is therefore proposed that a single adduct is generated but higher reagent ratio is required for optimum detector response. The different steps leading to the formation of the acid decomposition products may have required the higher reagent concentrations for ART and ATM. The result of the stoichiometric ratio determination is presented in Figure 4.

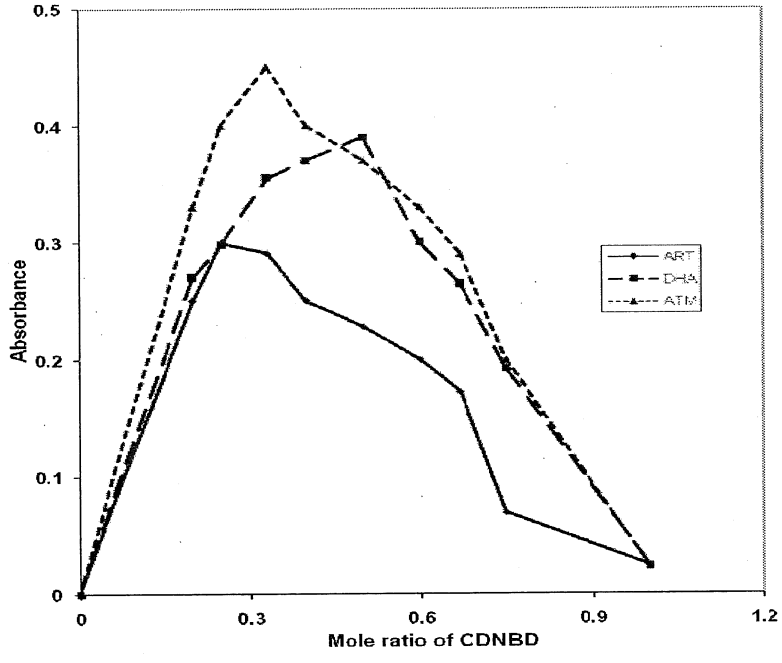


Figure 4. Determination of stoichiometric ratio for reagent-drug adduct formation

Validation studies

The various analytical and validation parameters are presented in Table 2. The regression equation for the calibration lines are; ART ($y = 0.01842x + 0.1839$, $r^2 = 0.9972$), DHA ($y = 0.02324x + 0.3329$, $r^2 = 0.9958$) and ATM ($y = 0.0181x + 0.2808$, $r^2 = 0.9968$) with ranges 3.6-28.8, 5.2-26 and 5.4-21.6 µg/mL respectively. Low limits of detection were obtained for the three compounds; 0.95, 0.17 and 0.55 µg/mL respectively. Apparent molar absorptivities are: ART ($9.935 \pm 0.05 \times 10^3$ $Lmol^{-1}$ cm^{-1}), DHA ($1.209 \pm 0.091 \times 10^4$ $Lmol^{-1}$ cm^{-1}) and ATM ($9.524 \pm 0.030 \times 10^3$ $Lmol^{-1}$ cm^{-1}). The Sandell sensitivities obtained

indicate the high sensitivity associated with this new procedure for the spectrophotometric determination of artemisinin derivatives.

Table 2. Analytical and Validation parameters for the azo adducts of artemisinin derivatives with CDNBD

Parameter	Artesunate	Dihydroartemisinin	Artemether
Beer's law limits, (μgmL^{-1})	3.6 - 28.8	5.2 – 26.0	5.4 – 21.6
Limit of detection, (μgmL^{-1})	0.95	0.17	0.55
Limit of quantitation, (μgmL^{-1})	2.85	0.51	1.65
Molar absorptivity ($L\ Mol^{-1}\ cm^{-1}$)	9.93×10^3	1.21×10^4	9.52×10^3
Sandell's sensitivity, (ng cm^{-2} per 0.001 absorbance unit)	542.9	431.0	552.5
Regression equation[a]			
Intercept, a	0.1839	0.3343	0.2808
Slope, b	0.01842	0.02321	0.0181
Correlation coefficient, r	0.9972	0.9964	0.9984
Confidence interval of intercept, α	0.0222	0.03305	0.01393
Confidence interval of slope, β	0.0005541	0.0009599	0.01146

Evaluation of the accuracy and precision of the new method was determined for three consecutive days. The inter-day recoveries obtained are presented in Table 3. The recoveries for ART ranged from 96.26 to 102.91%, DHA (96.49- 101.91%) and ATM (97.98 – 101.70%). The high recoveries obtained coupled with low relative errors over the three-day assessment periods lend credence to the suitability of this new procedure.

Table 3. Inter-day Accuracy and Precision for the proposed method

Drug	Taken ($\mu g\ mL^{-1}$)	Recovery [a]	RSD (%)	RE (%)
ART	7.2	99.26	1.69	-0.75
	14.4	96.19	0.1123	-3.96
	21.6	102.91	1.412	+2.83
DHA	7.8	101.24	1.625	+1.225
	15.6	101.91	1.089	+1.874
	20.8	96.49	0.617	-3.64
ATM	5.4	100.23	0.534	+0.23
	13.5	101.70	0.703	+1.67
	18.9	97.98	0.367	-2.06

[a] *n=12, overall recovery for ART is 99.45± 2.74(RSD % = 2.76); DHA is 99.88 ± 2.41 (RSD % = 2.41) and ATM is 99.97 ± 1.53 (RSD % = 1.53)*

Analytical application of the new method

The new procedures developed for the three artemisinin derivatives were thereafter applied to the determination of these derivatives in various dosages, mainly 4 tablet brands of artesunate, 3 tablet brands of DHA and 4 tablets, one injectable dosage forms of ATM. The results obtained are presented in Table 4. The new CDNBD method compared favourably with the International Pharmacopoeia methods for the assay of these dosage forms. There was found to be no significant difference in the content of each drug in the dosage forms ($p>0.05$). The method also successfully determined the dosage forms in the presence of additional analyte signifying the applicability of the procedure in workable range of the calibration curve. However, during the assay of artemether tablet brands containing lumenfantrine, a

partial overlap of the absorption spectra of lumenfantrine was observed on the spectra of artemether. The absorbance values were resolved as suggested by Olaniyi (2000).

Table 4. Determination of Artemisinin derivatives in dosage forms using the new and IP methods*

Drug formulation	New Method[a] (%±SD)	95% C.I.[b]	% found with additional analyte	Official Method	p-value[a] F-test	t-test
Artesunate						
Lever	101.65±1.12	14.64 ± 0.90	100.96 ± 1.64	104.57±1.14	0.46	0.95
Artesunat Tablet	104.85±1.10	15.10 ±0.88	105.32± 2.29	102.76 ±1.05	0.14	0.41
Askasunate Tablet	104.57±1.57	15.06 ±1.26	105.95±0.55	106.48±1.29	0.11	0.06
Malmether Tablet	96.08±1.09	13.80 ±0.87	99.95± 0.55	98.42 ±1.82	0.21	0.08
Dihydroartemisinin						
Cotecxin Tablet	99.44±1.54	15.51 ±1.23	99.24 ± 1.32	99.32 ± 1.11	0.36	0.96
Alaxin Tablet	96.83 ± 1.61	15.11 ± 1.23	95.52 ± 0.36	97.95 ± 1.62	0.46	0.53
Temexcin Tablet	70.32 ± 1.51	10.97 ± 1.21	72.12 ± 0.28	74.31 ± 1.72	0.28	0.03
Artemether						
Lonart Tablet	104.05±1.11	11.24±0.89	105.59±0.83	101.47±1.52	0.61	0.37
Artem Tablet	97.13± 1.74	10.49±1.39	93.27±0.71	98.51±1.77	0.55	0.01
Artrim Tablet	99.06±1.78	10.70±1.42	98.38±1.39	102.23±0.67	0.25	0.28
Arcofan Tablet	101.44±1.38	10.96±1.10	102.93±1.80	103.28±1.02	0.93	0.34
Gvither Injection	103.02±1.22	11.13±0.98	101.71±1.44	102.71±1.52	0.94	0.54

** Content of ART, DHA and ATM stipulated by IP = 90 – 110% (95 – 105 % for ATM injection); [a] n=6; [b] confidence interval calculated for 14.4 (ART), 15.6 (DHA) and 10.8 μg mL^{-1}ATM; [c] statistical analyses done between the results obtained from the proposed method and the official methods*

Assessment of interference from excipients

The effect of common excipients and substances were tested for possible interference in the assay. It was observed that starch, magnesium stearate, gelatin, lactose, talc and a mixture of these excipients did not interfere with the determination at the level found in dosage forms. This, therefore, implies that none of the drug will need prior extraction before spectrophotometric assay is carried out. The result of the interference assessment is presented in Table 5.

Table 5. Interference studies of commonly used excipients [a]

Excipient	Recovery (%)[b] ART	DHA	ATM
Starch	98.89 ± 0.24	100.23 ± 0.29	102.25±1.15
Magnesium stearate	99.06 ± 0.12	98.14 ± 0.13	96.10±0.50
Gelatin	98.19 ± 0.62	99.42 ± 0.06	101.05±1.85
Lactose	101. 62 ± 0.20	101.61 ± 0.26	96.25 ± 0.35
Talc	99.70 ± 0.72	100.14 ± 0.90	99.15 ± 0.25
Mixture	99.20 ± 0.38	100.90 ± 0.13	100.95 ± 0.45

[a] *Concentration spiked= ART (14.4 μg mL^{-1}), DHA (15.6 μg mL^{-1}) and ATM (10.8 μg mL^{-1})*
[b] *Mean ± S.D.; n=4*

The new procedure carried out in this report is simple, accurate, precise and economical and uses small sample size. The new procedures for artesunate, dihydroartemisinin and artemether could find application in the in-process quality control of the drugs in pure and in dosage forms. It is the first comprehensive report on the spectrophotometric determination of artemisinin derivatives.

Conclusions

In the present study, 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) was successfully applied to the assay of artesunate, dihydroartemisinin and artemether in bulk and dosage forms. The procedure gave

accurate and precise results and it is less time-consuming than previously reported methods for the quantitation of artemisinin derivatives. The commonly used tablet excipients did not interfere in the analysis. The proposed method is also sensitive. It could find application in the quality control of these drugs in bulk and dosage forms.

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