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New and sensitive spectrophotometric methods for the determination of cardol-an agriculture by-product

Masoumeh Moghimi, Akheel Ahmed Syed*
Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore-570 006, (INDIA)
Tel : +91-0821-2419660
E-mail: akheelahmed54@rediffmail.com

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ABSTRACT

Cardol is a phenolic compound found in cashew (*Anacardium occidentale*) shell liquid(CNSL), which is a by-product of cashew processing industry. It holds considerable promise for use because of its large availability in tropical areas, low cost, biodegradability and structural characteristics. Three simple, sensitive, selective, rapid and reliable spectrophotometric methods for the determination of cardol, an agriculture by-product have been developed. Method A describes the reaction of cardol with iron(III) and subsequent reaction with ferricyanide to yield a Prussian Blue product with maximum absorption at 760nm. Method B is based on the interaction of cardol with fast red B salt(FRS) at pH 9.6 to produce an orange colored product having maximum absorbance at 440nm. In method C a study was made of the application of diazotization-coupling spectrophotometric technique for the determination of cardol, using metaclopramide (MCP) and sulphanilic acid in alkaline medium to produce a yellow colored product having maximum absorption at 430nm. These methods obey Beer’s law. Ten independent anions and cations tried with these methods did not interfere. © 2008 Trade Science Inc. - INDIA

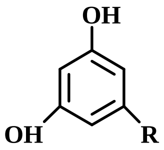
KEYWORDS

Spectrophotometry;
Cardol;
Prussian Blue;
Agriculture by-product;
Cashew nut.

INTRODUCTION

Cashew nut(*Anacardium occidentale*) shell liquid (CNSL), a by product obtained during the processing of cashew nut, is used in the manufacture of industrially important products such as cement, specially coating, primers, etc.[1]. The major phenolic constituents of CNSL are 70% anacardic acid, 18% cardol, 5% cardanol and other constituents include 2-methyl cardol and small amount of polymeric material.

The oily extract obtained by roasting cashew nuts (CNSL) is one of the most important sources of cardol and methylcardol, which are used in chemical formaldehyde polymerization and production of resins inindustry[1,2]. Cardol and methylcardol (trivial names) belong to the group of natural and easily isolate amphiphilic compounds[3] and are homologs of orcinol (1,3-di-hy droxy-5-methylbenzene) or methylorcinol (1,3-dihydroxy-2-methyl-5-methylbenzene) with C_{15} alkenyl side chain attached to the ring instead of 5-methyl group, that is predominantly di- and tri-enoic(double bonds at C 8, 11, 14) (Figure 1). These compounds due to their amphilic properties get incorporated very fast and effectively incorporate into the phospholipids bilayers exhibiting effects[4-10] that were dependent on their incorporation[9,11,12].



1. R=8Z, 11Z, 14 Pentadecatrienylmol. Wt.:299.43
2. R=8Z, 11Z Pentadecadienyl Mol. Wt.:301.45
3. R-8Z Pentadecenyl Mol. Wt.:303.46
4. R=Pentadecyl Mol. Wt.:305.43

Figure 1: Structure of cardol

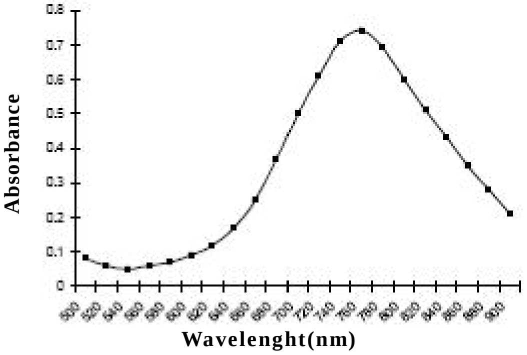


Figure 2 : Absorption spectrum of cardol-iron(III)-potassium ferricyanide

Cardol holds considerable promise in its diversified use because of the abundant availability in tropical areas, low cost, biodegradability[13] and structural characteristics[14]. The non-linear structure, unsaturation in the alkyl chain and substitution to phenolic group makes it amenable for innumerable applications including dyestuffs, foods, flavors, ion exchange resins, paints, plasticizers and polymers[15]. Significant progress has been made in the technological applications of cardanol and its derivatives mainly as pesticides[16], surface-active agents[16], in ceramics[14] and composites[13]. Cardanol is another phenolic compound present along with cardol in the CNSL with one OH group. Since cardol has two OH groups it is possible that this compound will have more versatile applications than its counter part cardanol.

In this paper we report simple, sensitive, rapid and reliable spectrophotometric methods for the determination of cardol using some pharmaceuticals as new coupling agents.

MATERIALS AND METHODS

Reagents and apparatus

Stock solution (100μg ml^{-1}) of cardol was prepared by dissolving in isopropyl alcohol in 100ml volumetric flask and made up to mark. Solution of the required strength was prepared by diluting this stock solution with isopropyl alcohol. Cardol from Vital Mallya Scientific Research Foundation, India, and metaclopramide (MCP) from Ipca Labratories Ltd. (India) were obtained as gift samples.

Method A (Prussian blue): The 0.3%(w/v) solution of iron(III) chloride containing few drops of 2N hydrochloric acid and 0.1% (*w/v*) potassium ferricyanide was prepared using double distilled water.

Method B: Aqueous solution(100μg ml^{-1}) of Fast Red B salt (FRS) was prepared in double distilled water. The pH was adjusted using sodium bicarbonate(0.05N) and sodium hydroxide (0.1N) solutions.

Method C: Aqueous solutions 1.0%(w/v) of sodium nitrite, 1.0%(w/v) sulphamic acid and 0.5N sodium hydroxide were prepared in distilled water. The 0.1%(w/v) solutions of MCP and sulphanilic acid were prepared by dissolving 100mg of each and diluting to 100ml with water. Solutions of diverse ions with the required strength were prepared by dissolving their corresponding salts in distilled water. Specord 50 UV-vis spectrophotometer with 1.0cm silica quartz matched cell was used for recording the absorption spectrum and absorbance measurements.

Procedure

Method A: Required volumes of standard solution of cardol, 1ml of iron(III) chloride 0.3% (w/v) and 1.5 ml of potassium ferricyanide 0.1%(w/v) were added to a series of 25ml calibrated flasks. The contents were mixed thoroughly and kept aside for 45min to complete the reaction. The resultant green coloured solutions in the standard flasks were made up to the volume with distilled water. Absorbance was measured against the corresponding reagent blank at 760nm (Figure 2). The concentrations of cardol as determined along with its optical characteristics are presented in TABLE 1.

Method B: Appropriate volumes of standard solution of cardol, 2 ml of FRS(100 μg ml^{-1}) and 4 ml of buffer solution (pH 9.6) were added to a series of 25ml calibrated flasks. The contents were mixed thoroughly and kept aside for 10min to complete the reaction. The resultant orange coloured solutions in the standard flasks were made up to the volume with distilled water. Absorbance was measured against the corresponding reagent blank at 440nm (Figure 3). The concentrations of cardol as determined along with its optical characteristics are presented in TABLE 1.

Method C: One ml of sulphanilic acid or MCP 0.1% (w/v), 1ml of sodium nitrite 1.0% (w/v), 0.5ml of sulphamic acid 1.0%(w/v), appropriate volumes of standard solution of cardol and 0.5ml of 0.5N sodium hydroxide were added to a series of 25ml calibrated flasks. The contents were mixed thoroughly and kept aside for 10min to complete the reaction. The resultant yellow colored solutions in the standard flasks were made up to the volume with distilled water. Absorbance was measured against the corresponding reagent blank at 430nm (Figure 4). The concentrations of cardol as determined along with its optical characteristics are presented in TABLE 1.

RESULTS AND DISCUSSION

Method A: The reaction of cardol with iron(III) salts in the presence of potassium ferricyanide in neutral medium formed a prussian blue (PB) product. The green pigmented iron(III) hexacyanoferrate(II) has been produced for many decades for use as a photo stable material for making inks, paints, lacquers and the like[17]. In the recent past, other works described include, dopant in modified electrode[18], composite films and code position of PB with polypyrrole[19]. Besides, PB has been extensively used as electrochemical sensor[20], biosensor[21,22], ion sensor[23] and as chemical resistor in the determination of alkaline metals[24]. Spectral[25-30] and volta metric studies[31,32] describe the structure, configuration and properties of PB.

Addition of a few drops of 2N HCl is necessary to prevent precipitation of iron(III) as hydrated ferric oxide. Besides, the addition of hydrochloric acid will bring down the pH of the solution to slightly acidic range.

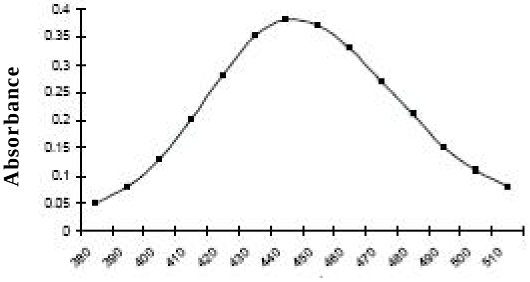


Figure 3 : Absorption spectrum of cardol-FRS

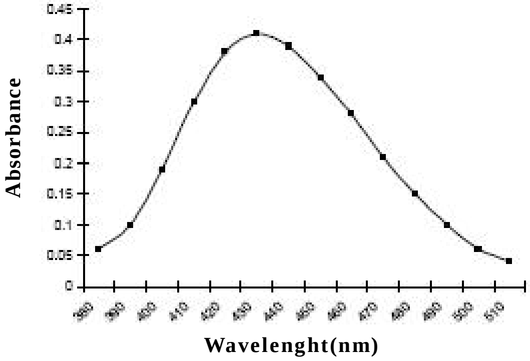
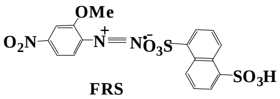
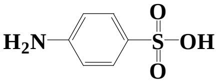
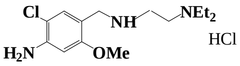


Figure 4: Absorption spectrum of cardol-MCP

TABLE 1: Optical characteristic for the determination of cardol l.c

Parameters	Method A	Method B	Method C	
	PB	 FRS	 **Sulphanilic acid**	 metaclopramide l.c
Colour	Green	Orange	Yellow	Yellow
λ_{max} (nm)	760	440	430	430
Stability (h)	3	2	6	6
Beer's law range (μg ml^{-1})	0.05-0.40	0.40-5.20	1.00-5.50	0.90-6.00
Recommended concentration(μg ml^{-1})	0.14	2.20	2.50	2.40
Molar absorptivity(L mol^{-1}cm^{-1})	5.90×10^{5}	4.09×10^{4}	3.40×10^{4}	3.60×10^{4}
Sandell's sensitivity(μg cm^{-2})	5.10×10^{-4}	7.38×10^{-3}	8.80×10^{-3}	8.40×10^{-3}
Regression equation[a]:				
Slope 'a'	0.1240	0.111	0.1366	0.1304
Intercept 'b'	-0.0295	0.0344	-0.0441	-0.0245
Correlation coefficient 'r'	0.9848	0.9886	0.9961	0.9867

[a]Regression curve: y =ax + b where x is the concentration in μg ml^{-1} of cardol and y is absorbance, Methods A, B and C as in test; PB: Prussian Blue; FRS: Fast Red B salt

Neutral ferric chloride free from hydrochloric acid is reported to give different colors like green, and purple or blue which have been extensively exploited as a confirmatory test of phenols in organic chemistry[33].

Method B: FRS is a diazonium salt often recommended for demonstrating enterochromaffin in characinoid tumors. In this method coupling of diazonium salt with cardol (active hydrogen) takes place at pH 9.6, which produces an orange colored dye having maximum absorption at 440nm.

Method C: Aromatic diazonium ions couple with active substrates such as amines and phenols[34-36]. Because of the size of the attacking species, substitution mostly takes place para to the activating group. If that position is already occupied then ortho substitution takes place. In case of cardol, the substituents being in the meta position, the substitution occurs preferably in the para position.

The pH of the media is of paramount importance for the activation of the substrates. Alkaline medium is recommended for phenols, because phenols are not active enough for the diazotization reaction. Nevertheless, there is a risk of unstable derivatives and large values of blank results due to the process of hydroxy-dediazonization[37], which reacts with excess of the reagent in basic medium.

Spectral data

The reproducibility, sensitivity and adherence to Beer's law with respect to color development were investigated for each reagent separately. TABLE 1 shows the linear calibration ranges and equation parameter for each method. Separate determinations at differentconcentrations of each reagent gave coefficient of variation not exceeding 2%. The reaction was carried out at room temperature. The intensity of green, orange and yellow colors was stable up to 3, 2 and 6h in method A , B and C, respectively. The proposed methods have distinct advantages of sensitivity and stability. Besides, the methods do not require heating or distillation and exhibit reliability due to reproducibility.

Order of addition of reagents

It was observed during the study that the sequence of addition of reactants also influences the intensity of colour and the stability of the coloured product. In method A the addition of reactants in sequences (i) iron (III) chloride-ferricyanide-cardol and (ii) ferricyanide-cardol-iron (III) chloride produced less intense and unstable green color, while (iii) cardol-iron(III)chloride - ferricyanide sequence gave more intense and stable green color. Hence, this sequence was selected for further work.

In Method B the sequences (i) FRS-buffer- cardol and (ii) buffer-cardol-FRS gave less intense and unstable orange color, while the sequence (iii) cardol-FRS-buffer gave more intense and stable orange color. Therefore, it was used in all further works.

In Method C the sequences (i) cardol-MCP or sulphanilic acid- sodium nitrite -NaOH-sulphamic acid and (ii) MCP or sulphanilic acid-sodium nitrite-sulphamic acid-NaOH-cardol gave less intense and unstable orange color, while (iii) MCP or sulphanilic acid -sodium nitrite-sulphamic acid-cardol-NaOH gave more intense and stable yellow color. Hence, it was selected for further study.

Effect of temperature

The color development was not influenced by temperature in the range of 20-35^{0}C and gave the optimum results at room temperature. At higher temperature the absorbance values decreased and this is due to the dissociation of the coloured product on prolonged heating. Therefore, all the experimental works were carried out at room temperature.

Effect of reaction time

Experiments were conducted to optimize the duration needed in the determination of cardol. It was found that the green, orange and yellow colors formed in the reaction were not affected after 45 min with method A and 10min with method B and C, respectively and the colors in the respective methods remained constant up to 3, 2 and 6 h.

Optimization of analytical variables

Method A: Effect of different concentration levels of iron(III) chloride was studied using the concentration ranges from 0.05 to 0.5% of iron(III) chloride. Maximum absorbance was obtained at 0.3% of iron(III) chloride. Effect of different volumes of iron(III) chloride was studied in the range of 0.5-5.0ml. Maximum absorbance of the colored product was

TABLE 2: Effect of addition of cations on the determination of cardol by the proposed methods

Cation salt		% Recovery of cardol*±RSD**		
Name	**Quantity mg**	**Method A**	**Method B**	**Method C**
Barium sulphate	100	100.2±0.70	99.9±1.00	10.1.2±0.60
Cadmium sulphate	100	99.8±0.80	101.2±0.90	100.13±0.90
Copper sulphate	100	98.6±1.00	98.7±1.00	97.80±0.30
Lead nitrate	500	101.6±0.50	100.6±0.70	99.60±0.30
Magnesium sulphate	250	99.6±0.70	102.0±0.80	101.36±0.80
Manganese sulphate	100	97.4±0.60	97.6±1.10	97.00±0.70
Potassium dichromate	100	99.8±0.70	98.7±0.50	100.4±1.00
Strontium nitrate	100	98.6±1.00	101.3±1.10	101.0±0.50
Tin chloride	100	100.1±0.90	100.4±0.90	99.30±0.40
Zinc sulphate	100	101.4±1.00	98.6±0.90	99.70±0.50

***0.14mg $ml^{-1,}$ 2.2μg ml^{-1} and 2.4μg ml^{-1} of cardol taken for Method A, B and C respectively; **Relative standard deviation (n=5)**

TABLE 3: Effect of addition of anions on the determination of cardol by the proposed methods

Anion salt		% Recovery of cardol*±RSD**		
Name	**Quantity mg**	**Method A**	**Method B**	**Method C**
Ammonium phosphate	200	100.2±1.00	101.6±0.80	101.0±0.80
Calcium carbonate	500	100.6±0.60	101.3±0.90	100.2±1.30
Potassium braomate	100	98.9±0.80	102.8±0.80	99.70±0.70
Potassium chloride	250	98.4±0.70	100.8±0.70	100.7±0.80
Potassium iodate	100	101.5±1.10	99.9±0.50	98.60±0.80
Potassium sulphate	100	100.8±1.00	97.8±1.00	101.2±0.30
Sodium fluoride	100	102.1±0.60	101.8±1.00	100.7±0.60
Sodium nitrate	100	98.7±0.90	99.6±0.70	100.6±0.40
Sodium phosphate	100	99.8±0.70	100.4±1.00	99.60±0.40
Sodium sulphate	100	99.6±1.00	98.2±0.90	97.00±0.80

***0.14mg $ml^{-1,}$ 2.2μg ml^{-1} and 2.4μg ml^{-1} of cardol taken for Method A, B and C respectively; **Relative standard deviation (n=5)**

noticed when 0.5-1ml of 0.3% iron(III) chloride was used. Therefore, 1ml of 0.3% (w/v) iron(III) chloride was used for further study.

Similarly, the above procedure was repeated to ascertain the amount of ferricyanide required for getting constant and maximum color intensity. The results showed that 0.50-5.00ml of the solution were needed to get good result. Hence, 1.5ml of(0.1%w/v) ferricyanide solution was found sufficient to get reproducible results.

Method B: FRS was studied in the range of 1-5ml (100μg ml^{-1}) solution to find out the maximum color intensity; 1-3ml of the solution gave good result. Hence, 2ml (100μg ml^{-1}) solution in 25-ml standard flask was selected for further studies, to get optimum results. As the maximum intensity of the orange was achieved in buffer medium. It was selected in the range of 1-5ml with pH 9.6. Therefore, 4ml of buffer solution (pH 9.6) in 25 ml were used for getting the best results.

Method C: In the case of cardol, 1-3ml sodium nitrite (1.0% w/v), 1-2ml sulphamic acid (1.0% w/v) and 0.5-3ml 0.5N sodium hydroxide gave reproducible results. Hence, sodium nitrite and sulphamic acid each at 1ml and 0.5ml sodium hydroxide were found appropriate. Experiments carried out to find out the amount sulphanilic acid or MCP needed showed that 1-2ml of 0.1% (w/v) sulphanilic acid or MCP 0.1%(w/v) produced maximum color intensity. Hence, 1ml of sulphanilic acid or MCP 0.1%(w/v) was found appropriate.

Analytical data

Linearity of the green colored species obtained range from 0.05 to 0.40μg ml^{-1} in method A. Molar absorptivity values were 5.90×10^{5} $Lmol^{-1}cm^{-1}$. Sandell's sensitivity value as calculated from Beer's law was found to be 0.00051μg cm^{-2}. Regression analysis of Beer's law gave a correlation coefficient value of 0.9848. Low intercept (-0.0295) and slope(0.1240) values were found in method A. The apparent molar absorptivities, correlation coefficients, intercept and slopes, for the calibration data are presented in TABLE 1. The values in the TABLE show negligible intercept as determined by the regression equation, $Y=ax+b$

Effect of diverse ions

Interferences of foreign species in the analysis were investigated by analyzing 0.14 μg ml^{-1}, 2.20 μg ml^{-1} and 2.40 μg ml^{-1} standard solution of cardol for method A, B and C respectively to which increasing amounts of likely interfering species salts of cations (TABLE 2) and anions (TABLE 3) were added. The tolerable limit of a foreign species was taken as a relative standard deviation not greater than ±3%. It was also found that

TABLE 4: Recovery of cardol in the presence of excipients in Methods A, B and C

Material	Amount (mg)			(%) Recovery of cardol* ±RSD**		
	Method A	Method B	Method C	Method A	Method B	Method C
Glucose	50	50	40	100.3±1.25	100.4±0.65	97.2±0.82
Lactose	50	50	50	98.6±0.68	99.7±1.05	100.2±0.52
Dextrose	30	10	10	99.7±0.85	97.4±0.89	99.40±0.57
Starch	70	5	5	100.1±0.78	100.6±0.69	100.0±0.41
Sucrose	50	10	30	99.9±0.69	97.1±0.90	100.7±0.73
Maltose	30	5	5	99.0±0.91	99.8±0.96	99.60±0.59
Gelatin	30	10	10	100.8±0.82	100.7±0.58	100.2±0.21

***0.14, 2.2 and 2.4mg ml of cardol taken for method A, B and C, respectively; **Relative standard deviation (n=5)**

excipients such as glucose, lactose, dextrose, starch, gelatin, sucrose and maltose did not interfere, while vitamin C was found to interfere in the analysis of cardol when tested as per the methods A, B and C(TABLE 4). In method C, MCP was used for the analysis as it was found to be more sensitive than sulphanilic acid.

CONCLUSION

One of the recent frontiers of sustainable development has been the utilization of agriculture by-products. Effective utilization of any product is based on its quality, which, in turn, depends on the analytical data. Method A standardized for cardol determination has distinct advantages of sensitivity and reproducibility. The use of aqueous neutral medium and easily available chemicals make the procedure cost-effective and easily adaptable.

Methods B and C described here make use of diazonium ions as spectrophotometric reagents in the determination of cardol-a phenolic compound found in agriculture by-product cashew nut shell liquid. Two important dimensions of this study include; first, the success in finding new spectrophotometric reagents amongst the available myriad molecules in the field of pharmaceuticals, which has a variety for the functional groups and molecular structure. Second, it will open up a new area of research on the dyes produced in the reaction of cardol with diazonium ions. Another dimension to this method could be achieved if the procedure is made on-line or at-line system and this possibility is currently under investigation.

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