FLOW INJECTION DETERMINATION OF SALBUTAMOL USING A SOLID-PHASE REACTOR CONTAINING LEAD (IV) DIOXIDE IMMOBILIZED

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ABSTRACT:
A simple, sensitive and fast continuous flow injection system with spectrophotometric detection was proposed for the determination of salbutamol sulphate (SAL) in pharmaceutical formulations using a solid-phase reactor containing PbO_2 immobilized in a polymeric matrix. The method was based on oxidation of the reagent (N, N-dimethyl-p-phenelynediamine) with PbO_2 producing dimethylbenzoquinone diimine, which is then coupled with SAL in alkaline medium. The blue colored product was monitored at 640 nm. The calibration graph was linear over the range 0.5 to 60 µg mL^{-1} with a relative standard deviation less than 3.8% (n=33) and sample throughput 60 samples per hour. Solid-phase reactors can be applied to the determination of SAL in a flow injection system with high sensitivity and significant advantages over conventional procedures.
Keyword: Reverse-flow injection, Salbutamol sulphate, spectrophotometric.

1. Introduction

Salbutamol sulphate (SAL), chemically known as bis[(1RS)-2-[(1,1-dimethylethyl) amino]-1 -[4-hydroxy-3-(hydroxymethyl) phenyl]ethanol] sulphate, is an agonist for β_2 receptor which are present in the bronchioles of lungs of human body and therefore acts as a bronchodilator and its cardiovascular effects are less than its bronchodilator actions[1]. Different methods of analysis have been reported for the determination of SAL including HPLC[2,3], capillary electrophoresis[4], voltammetry[5], flow injection analysis[6] and titrimetric[7].

In the present work a solid-phase reactor coupled with flow injection system is adopted for estimation of SAL. This strategy has many advantages such as possibility to use reagents that are not available in soluble form and subsequently avoid the time consuming steps for preparation of reagent solutions. For the first time an oxidative coupling reaction between SAL and *N*,*N*-dimethyl-*p*-phenelynediamine (DMPD) was adopted as a basis to develop a reverse flow injection (rFIA) method using solid phase reactor containing PbO_2 as oxidizing agent. The method is based on the oxidation of the reagent with PbO_2 immobilized on a polymeric matrix, which is then coupling with SAL in alkaline medium.

2. Experimental

2.1. Reagents and solutions

- Standard salbutamol sulphate solution (State Company for Drug Industries and Medical Appliance, SDI, Samara, Iraq): stock solution (1000µg mL^{-1}) was prepared by dissolving 0.1g of the pure drug in 100mL of distilled water and further diluted with the same solvent as appropriate.
- N,N-Dimethyl-p-phenylenediamine sulphate (BDH, UK): 5mM aqueous solution freshly prepared in distilled water.
- Ammonium hydroxide solution (Fluka, Buchs, Switzerland): 1 and 0.2 M aqueous solution.
- Dimethylformamide, Aceton and Cellulose acetate (BDH, UK).
- PbO_2 (Merck, Chemicals Ltd., Germany).

2.1.1. Preparation of solid -phase reactor containing PbO_2: The immobilization of PbO_2(s) was similar to that previously reported[8]. Cellulose acetate (CA) (0.5g) was dissolved completely in 0.5 mL of dimethylformamide and 3 mL of acetone solution with continuous stirring. This was followed by the slow addition of 4 g of lead dioxide powder to the solution. The mixture was homogenized by manual stirring until an obvious increase in viscosity was observed. Ten minutes later the homogenized mixture was washed with water and a rigid polyester solid was obtained. After air-drying, the polyester containing the immobilized PbO_2 was cut into 1.18 mm particles with a scissor. The solid phase reactor was prepared by packing the polyester particles into glass tubes of different lengths (2 mm i.d.). Small pieces of sponge were inserted at the ends of the tubes to hold the particles in place.

2.1.2. Solutions of pharmaceutical preparations

- ***Tablets samples:*** Twenty tablets were accurately weighed and finely powdered. An amount of the powder equivalent to 20 mg of SAL was dissolved in distilled water. The solution was filtered into a 50 mL volumetric flask and the residue was washed and diluted to the required volume with the same solvent to obtain 400 µg mL^{-1} of SAL.
- ***Syrup samples:*** The contents of three bottles of SAL syrup were mixed. An aliquot corresponding to 10 mg of SAL (25 mL) was diluted to 50 mL with distilled water in a volumetric flask to obtain 200 µg mL^{-1} of SAL. Other samples of SAL were prepared by simple dilution from the stock solution using the same solvent.

2.2. Apparatus: All spectral and absorbance measurements were carried out on a Shimadzu UV/VIS 260 digital double beam recording spectrophotometer. A flow cell with 50 µL internal volume and 1 cm bath length was used for the absorbance measurements. A two-channel manifold was employed for the rFIA spectrophotometric determination of SAL drug (Fig. 1). A peristaltic pump (Ismatec, Labortechnik Analytik, CH8152, Zurich, Switzerland) was used to transport the carrier solution. An injection valve (Rheodyne, Altex 210, Supelco, USA) was employed to provide appropriate injection volumes of the standard solutions and samples. Flexible vinyl tubing of 0.5 mm internal diameter was used for the peristaltic pump. Moreover, Teflon made reaction coil (RC) with an internal diameter of 0.5 mm was utilized.

The rFIA manifold contained two channels (Fig. 1). The reagent (DMPD) was injected into the stream of SAL solution through the injection valve which then oxidized through the solid phase reactor (8 cm, with particles size of 1.18 mm), then combined at Y-link with stream of ammonium hydroxide and mixed in RC. Solutions were propelled by peristaltic pump with a flow rate of 3.25 mL min^{-1} (1.63 mL min^{-1} in each line). The absorbance was then measured at 640 nm.

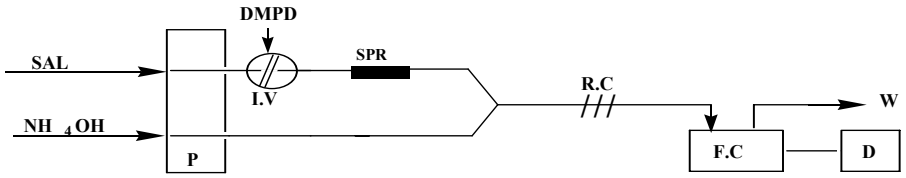
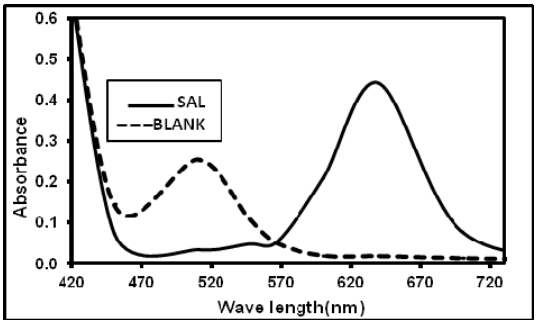


Fig.1: A schematic diagram of rFIA manifolds. P, peristaltic pump; IV, injection valve; RC, reaction coil; FC, flow cell; D, detector; W, waste; SPR, solid phase reactor; DMPD, N,N-dimethyl-p-phenylenediamine; SAL, salbutamol sulphate.

3. Results and Discussion

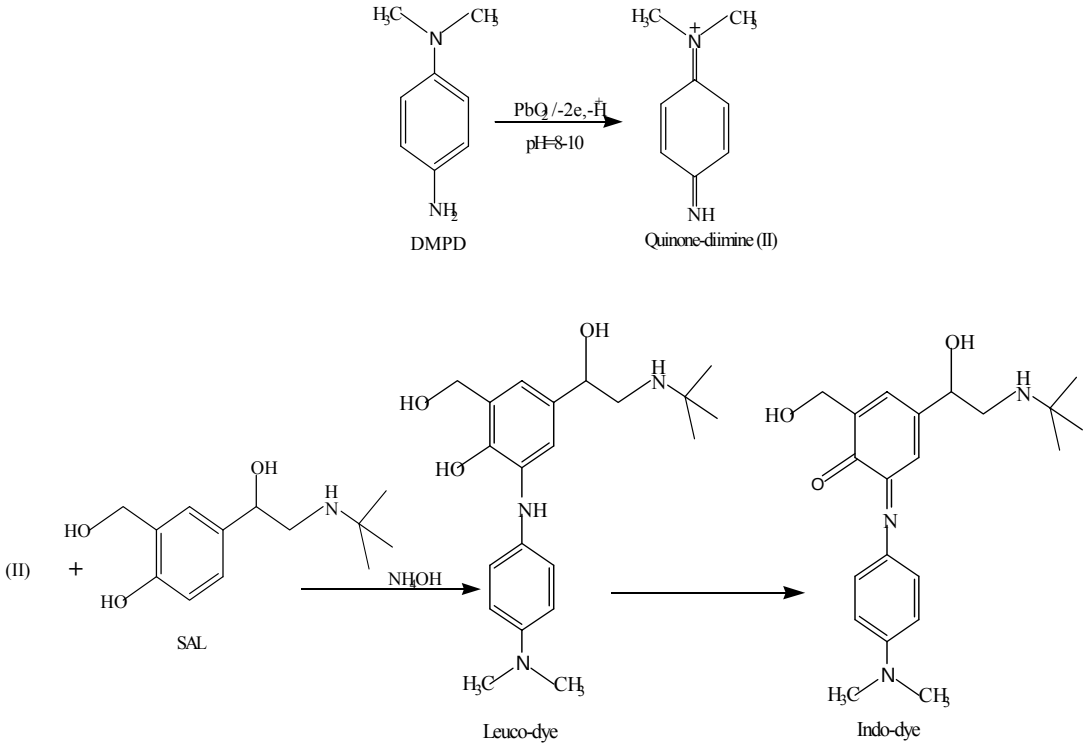
3.1. Preliminary studies: The preliminary investigation of the proposed reaction was carried out in a 25 mL volumetric flask. To a flask containing 50 µg mL^{-1} of SAL, a volume of 2 mL of 4 mM of DMPD, 2 mL of 0.5M of ammonium hydroxide and an amount of 0.03 g of PbO_2 immobilized on CA were added. The flasks were swirled immediately and made up to the volume with water and then filtered. The maximum absorption of the product was recorded at 640 nm (Fig. 2).

Fig. 2: Absorption spectra of 50 µg mL^{-1} of SAL.



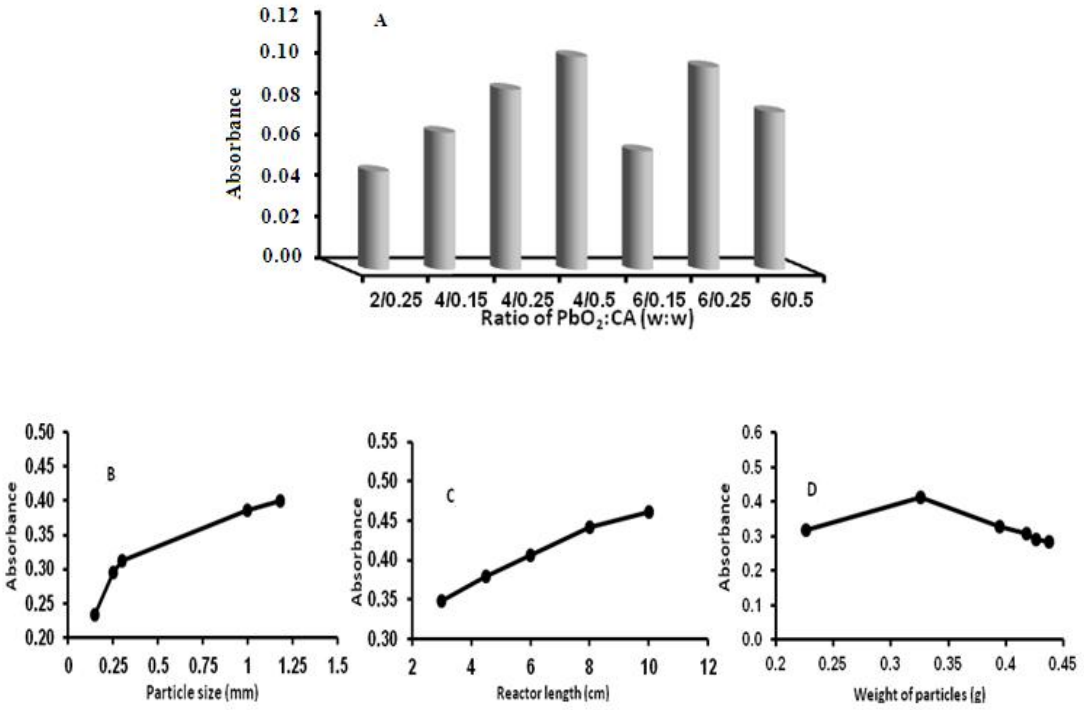
DMPD is oxidized by PbO_2 losing two electrons and a proton to yield reactive diethylbenzoquinone-diimine which couples with SAL (Fig. 3) by electrophilic attack at their nucleophilic site, preferably at ortho-position to give a leuco-dye, which is oxidized to an indo-dye [9].

Fig.3: Scheme of reaction mechanism for the formation of an indo-dye.



3.2. Solid phase reactor parameters: The lead dioxide solid-phase reactor is essential in this method. It is responsible for oxidation of DMPD into active dimethylbenzoquinone diimine compound as well as for dispersion of the reactant plug in the flow system. Therefore, the variables of the solid-phase reactor like composition, particles size and weight, and length of the reactor were optimized. The relationship between the absorbance and the amount of PbO_2 immobilized per unit mass of inert support (cellulose acetate) were established. Different PbO_2:CA ratios such as 2:0.25, 4:0.15, 4:0.5, 6:0.25, 6:0.5 and 6:0.15 (w/w) were examined. It was found that the ratio of 4:0.5 provides the highest absorbance and reproducibility (Fig. 4A). Small amounts of inert support (CA) should be avoided to ensure efficient immobilization of PbO_2 and to exclude partial solubility of the immobilized oxidant during the flow of the reagent. This may cause a decrease in the reproducibility and life-span of the reactor. The effect of the size of the particles was studied in the range of 0.15 to 1.18 mm (the reactor was filled with particles of different sizes but had the same weight of 0.1733 g). The results show that the absorbance has increased with any corresponding increase in the particles size. In addition, the smaller particles lead to a greater flow resistance therefore a particle size of 1.18 mm provided the highest absorbance and was selected for further experiments (Fig. 4B). The influence of the length of the reactor on the absorbance was examined within a range of 3 to 10 cm. The absorbance signal increased gradually with the increase in the reactor length (Fig. 4C). However, a reactor length larger than 8 cm showed baseline instability therefore 8 cm length was utilized for further experiments. The strong packing of solid-phase material causes an increase in the resistance against the flow of the solution which is propelled from the peristaltic pump and passes through the reactor. The degree of packing of solid-phase reactor was studied using different weights of the immobilized PbO_2 particles which had the same particle size (1.18 mm). The obtained results show that a 0.3260 g of solid-phase material gave the highest absorbance and was used in all subsequent experiments (Fig. 4D).

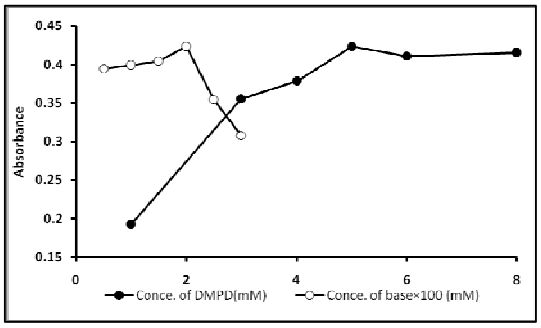
Fig.4: Effect of solid-phase compositions.



3.3. Optimization of flow-injection conditions: In order to increase the life-span of the reactor and decrease the consumption of the oxidant material immobilized on inert support (cellulose acetate) by continuous merging of reagent (DMPD) through the solid phase reactor, a reverse flow injection method was adopted rather than normal flow, in which a small volume of reagent would injected through the injection valve to the solid-phase reactor containing immobilized oxidant agent (PbO_2).

3.3 Effect of chemical parameters: The blue color of complex resulted from the reaction between SAL and DMPD in alkaline medium is relatively stable and its intensity is unusually high. The effect of different concentrations of DMPD (1 to 8 mM) was investigated (Fig.5). The results obtained indicated, that the absorbance increased with increasing concentration of DMPD up to 5 mM, thus a concentration of 5 mM gave the maximum absorbance and was chosen for further use. It was observed that the alkaline medium is very essential for the reaction between SAL and DMPD for developing the colored product and increasing its stability, therefore, the effect of different kinds of basic solutions (0.2M) were studied such as sodium carbonate, sodium acetate, sodium hydroxide and ammonium hydroxide. Maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of ammonium hydroxide solution .The effect of various concentration of ammonium hydroxide were studied in the concentration range of 50 mM to 0.3M and a greatest absorbance intensity was obtained with 0.2 M and was chosen for further use (Fig. 5).

Fig. 5: Effect of concentration of DMPD and ammonium hydroxide solutions

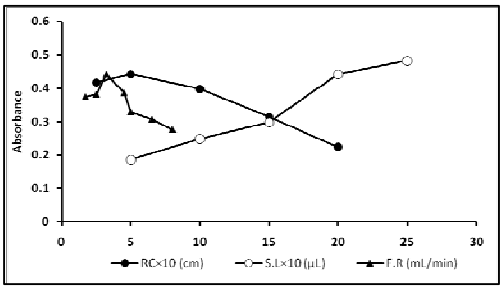


3.4 Effect of physical parameters: The physical parameters which are studied under the optimized reagent concentrations were the flow rate, the injected sample volume and the reaction coil length. The effect of the length of mixing coil was investigated in a range of 25 to 200 cm, at a length of 50 cm the absorbance reached to almost maximum then it decreased as the reaction coil was increased because of the increase in dispersion. Therefore, it could be suggested that the optimal length is 50 cm as shown in Fig. 6.

The effect of total flow rate (F.R) in the range of 1.75 to 8.0 mL min^{-1} was studied using equal flow rates of the two channels and using the optimum concentration of reactants. The absorbance increased with increasing of total flow rate up to 3.25 mL min^{-1} above which the absorbance decreased because of the dilution and increased dispersion. Thus, 3.25 mL min^{-1} (1.63 mL min^{-1} in each line) was regarded as the optimum flow rate (Fig. 6).

The volume of the injected sample was varied between 50 and 250 µL using different length of sample loop (S.L). A sample volume of 200 µL showed the best engagement between sensitivity and analytical frequency. Therefore, the injected sample of 200 µL was chosen and used in all subsequent experiments (Fig. 6). The optimum working conditions employed in this study were summarized in Table 1.

Fig. 6: Effect of physical parameters.



3.5 Sampling frequency and solid-phase reactor life-time: Under the optimum conditions, the sampling frequency was studied by recording the time from the sample injection to the maximum absorbance (60 sec). A sampling frequency of 60 per hour could be attained. The reproducibility and the life time of the reactor were investigated. It was found that the solid phase material was stable more than one month and the reproducibility of reactor preparation was good (RSD =3.511 for 38 injections) and with life time more than 48 injection (RSD≤5).

Table 1: The optimum conditions for the determination of SAL using solid-phase FIA.

Parameter	Studied range	Optimum value
DMPD conc. (mM)	1-8	5
Ammonium hydroxide conc. (mM)	50-300	200
Reaction coil (cm)	25-200	50
Total flow rate (mL/min)	1.75-8	3.25
Sample volume (µL)	50-250	200
Ratio of PbO_2:CA (g)	Different ratios	4:0.5
Reactor length (cm)	3-10	8
Particles weight (g)	0.2260-0.4372	0.3260
Particles size (mm)	0.15-1.18	1.18

3.6. Analytical characteristics: The regression equation obtained from a series of SAL standards and the analytical figures of merits of the flow procedure are summarized in Table 2. The quantification limit (10-fold blank standard deviation/slope) was 0.27 µg mL^{-1} and the detection limit (3-fold blank standard deviation/slope) was 0.89 µg mL^{-1} with relative standard deviation less than 2.6%.

Table 2: Analytical values of statistical treatments for the calibration graph.

Parameter	Value
Regression equation	Y=0.0157x+0.0458
Correlation coefficient, r	0.9994
Linearity range (µg mL^{-1})	0.5-60
Molar absorptivity, ε (L $mol^{-1}cm^{-1}$)	9.05×10^{3}
Slope, b (mL µg $^{-1}$)	0.015669
Intercept, a	0.045787
Standard deviation of the residuals, $S_{y/x}$	0.012506
Standard deviation of the slope, S_b	1.995×10^{-4}
Standard deviation of the intercept, S_a	6.187×10^{-3}
Through-put (h^{-1})	60
Limit of detection (µg mL^{-1})	0.267
Limit of quantification (µg mL^{-1})	0.891

3.7. Pharmaceutical applications: The proposed method was applied for the determination of SAL in tablets and syrup. This was performed by the ejection of three concentrations of the sample using optimum conditions in the rFIA manifold (Table 1). The recoveries were obtained at 97.1 to

102.4%. The statistical comparison between the recoveries obtained from proposed method with those of the pharmacopeial method revealed no significant difference between the accuracy and precision of both methods (Table 3).

Table 3: Application of the proposed method for determination of SAL in pharmaceutical forms.

Drug form	Proposed method (rFIA)				Official method Recovery (%)
	Conc. of SAL (µg mL^{-1})		Recovery%*	RSD%*	
	Present	Found			
- Aloprol®	20.00	20.48	102.39	2.47	99.500
(Tablets, 2 mg)	30.00	29.70	99.01	1.46	
- Butadin®	20.00	19.59	97.95	3.80	99.750
(Tablets, 2 mg)	30.00	29.68	98.92	2.87	
- Butadin® (Syrup,	20.00	20.31	101.56	2.96	101.000
2 mg /5 mL)	30.00	29.12	97.07	1.16	
t (2.57)**	0.77				
F(9.28)**	2.17				

*Average of five determinations, **Theoretical value

4. Conclusions

The catalytic oxidation of DMPD on the solid-phase reactor containing lead (IV) dioxide which then coupling with SAL drug is economical, fast and sensitive. It is free from most interfering substances and can be applied for the determination of SAL in pharmaceutical samples in the presence of other excipients. This study has demonstrated that the solid-phase reactors can be applied to the determination of SAL in a flow injection system with excellent sensitivity and significant advantages over conventional procedures. A wide linear range and good sample throughput were obtained (0.5-60 µg mL^{-1} and 60 h^{-1}) as compared with the batch method proposed by Nagaraja et.al.[10] (1-7 µg mL^{-1} and 6 h^{-1}) in addition the selectivity of the FIA-SPR method is attained without any sample pre-treatment or extraction the product as compared with normal FIA method used the same reagent with solution of KIO_4 as oxidant[11].

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