A VALIDATED SPECTROFLUORIMETRIC METHOD FOR DETERMINATION OF POLYSORBATE 80 FROM PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, rapid and sensitive spectrofluorimetric method has been developed and validated for the determination of the non-ionic surfactant, polysorbate 80, from pharmaceutical formulation. The proposed method is based on a fluorescence enhancement of the probe (eosin B dye) with addition of polysorbate 80. The eosin B concentration was optimised and found to be 4µg/mL. The fluorescence intensity was measured in a diluting solvent, citric acid buffer (pH 4.0) using excitation and emission wavelengths, 545 nm and 580 nm, respectively. The fluorescence intensity was found to be liner over a concentration range of 16-80 µg/mL of polysorbate 80 with a high correlation coefficient ($r = 0.9990$). The developed method was validated in terms of linearity, precision, accuracy, limit of detection and limit of quantification and specificity. The limit of detection and limit of quantification for polysorbate 80 were found to be 2 µg/mL and 16 µg/mL, respectively. The developed method was successfully applied for the determination of polysorbate 80 in ophthalmic solution and micro emulsion.

INTRODUCTION

Polysorbate 80, polyoxyethylene (20) sorbitan monoleate, is an amphiphilic nonionic surfactant and commonly known as Tween 80 (Fig. 1). It is typically used in pharmaceutical, food, cosmetic and biotechnological products[1]. Polysorbate 80 possesses high surface activity and low toxicity that makes it a good excipient for pharmaceutical applications[2]. Polysorbate 80 is widely used in liquid pharmaceutical formulations for solubilization of drug substance and stability of the product[3-4]. An optimal concentration of polysorbate 80 is required in pharmaceutical formulation. Determination of content of polysorbate 80 in pharmaceutical formulation is an important aspect of liquid pharmaceutical formulation development.

Polysorbates have heterogeneous molecular structures. In addition, the lack of a chromophore in polysorbate 80 make it difficult to analyze. Several methods are reported for the analysis of polysorbate 80 in pharmaceutical formulation. The reported methods include colorimetric[5], C^{13} NMR[6], SFC[7], HPLC-UV[8] after quantitative hydrolysis of polysorbate 80 and RP-HPLC with ELSD[9-11], CAD[11-14] or MS[10] detections. Since polysorbate 80 does not contain a UV chromophore, HPLC with absorbance detectors such as UV or PDA is not applicable for quantitative analysis. The chromatographic separation followed by detection with non-absorbance detectors such as ELSD, CAD or MS are reported for quantitative analysis of polysorbate 80[9-14]. The HPLC-ELSD and HPLC-CAD methods suffer from lack of specificity whereas HPLC-MS method is an expensive technique.

Spectrofluorimetric methods are generally characterized by highly sensitive, selective, rapid and simple analysis. Moreover, fluorescence spectroscopy is an adaptable analytical technique and is more sensitive than other detection systems such as classical ultraviolet (UV) absorption. Fluorimetric determination is time-saving compared with high-performance liquid chromatography (HPLC), and is less expensive than LC–MS/MS detection. Thus, fluorescence spectroscopy was used in this study.

The objective of the present work was to develop a simple, rapid, cost-effective and sensitive spectrofluorimetric method for determination of polysorbate 80 from pharmaceutical formulations. The proposed method is based on fluorescence enhancement of the probe 4',5'-dibromo-2', 7'-

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Table I: Intra-day and inter-day precision for the determination of polysorbate 80

Concentration of polysorbate 80 (µg/mL)	Intra-day precision		Inter-day precision	
	Fluorescence intensity (Mean±SD)	% RSD	Fluorescence intensity (Mean±SD)	% RSD
16	272±7.50	2.75	275±8.02	2.92
32	418±6.42	1.53	404±6.06	1.50
48	525±5.50	1.04	529±5.66	1.07
64	627±7.63	1.21	635±8.13	1.28
80	725±9.45	1.30	729±8.97	1.23

Table II: Accuracy of polysorbate 80 determination

% Level	Initial sample concentration (µg/mL)	Standard polysorbate 80 spiked (µg/mL)	Total concentration of polysorbate 80 (µg/mL)	Amount recovered (µg/mL)	% Recovery (Mean±SD)	% RSD
50	24	12	36	35.70	99.16±2.78	0.73
100	24	24	48	47.58	99.12±4.50	0.95
150	24	36	60	59.43	99.05±5.33	0.95

Table III: Content of polysorbate 80 in pharmaceutical formulations

Formulation	Actual amount (µg/mL)	Amount found (Mean ± SD) (µg/mL)	% RSD
Eye drops (marketed formulation)	50	49.07±1.81	3.69
Micro-emulsion (in-house)	15	15.16±0.62	4.09

dinitromfluorescein, disodium salt (Eosin B, Fig. 2) in the presence of a surfactant, polysorbate 80[15]. The dye-surfactant interaction is correlated with the surfactant concentration to allow development of a simple, rapid and sensitive method for determination of polysorbate 80 in pharmaceutical formulations.

MATERIALS AND METHODS

Instrumentation

Fluorescence spectroflurometer (LS 55, Perkin Elmer, UK) equipped with xenon discharge lamp (20KW) and two automatic monochromator, photomultiplier tube and FI Winlab software was used for recording fluorescence spectra. The pH measurements were made on a microprocessor laboratory pH meter (FE-20, Mettler Toledo, Zürich, Switzerland).

Materials

Polysorbate 80 and eosin B dye were purchased from Sigma-Aldrich, Bangalore, India. Sodium hydroxide, hydrochloric acid and citric acid AR were purchased from Loba Chemie, Mumbai, India. Milli-Q water was used throughout this work. Citric acid buffer solutions were freshly prepared. Eye drops containing polysorbate 80 (0.5%) was purchased from the local market. A micro-emulsion (in house formulation) composed of sapsol, polysorbate 80, carbitol and water was prepared in our laboratory.

STANDARD SOLUTIONS

Stock solution of polysorbate 80

Polysorbate 80 (20 mL) was transferred to 50 mL volumetric flask. It was diluted with water to obtain a stock solution of polysorbate 80 with final concentration of 400 mg/mL.

Working standard solution of polysorbate 80

Stock solution of polysorbate 80 (1 mL) was transferred to 10 mL volumetric flask and diluted with water up to the mark (40 mg/mL). Aliquot (1 mL) of resulting solution was transferred to 10 mL volumetric flask and diluted with water up to the mark to obtain working standard of polysorbate 80 with final concentration of 4 mg/mL.
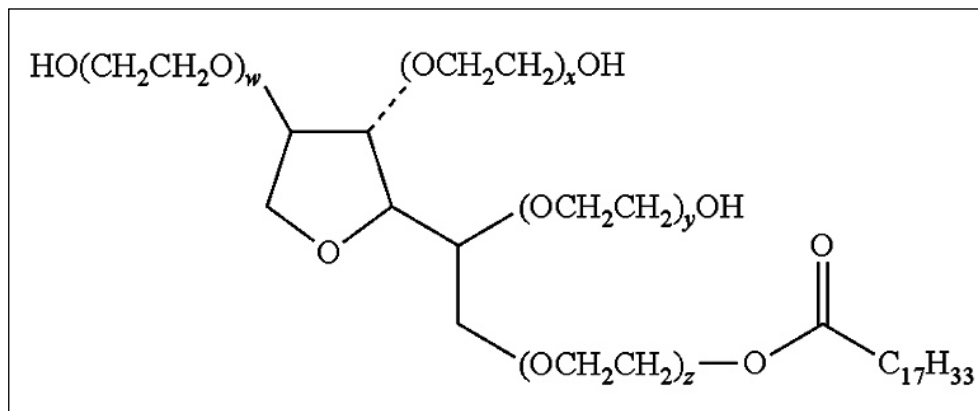
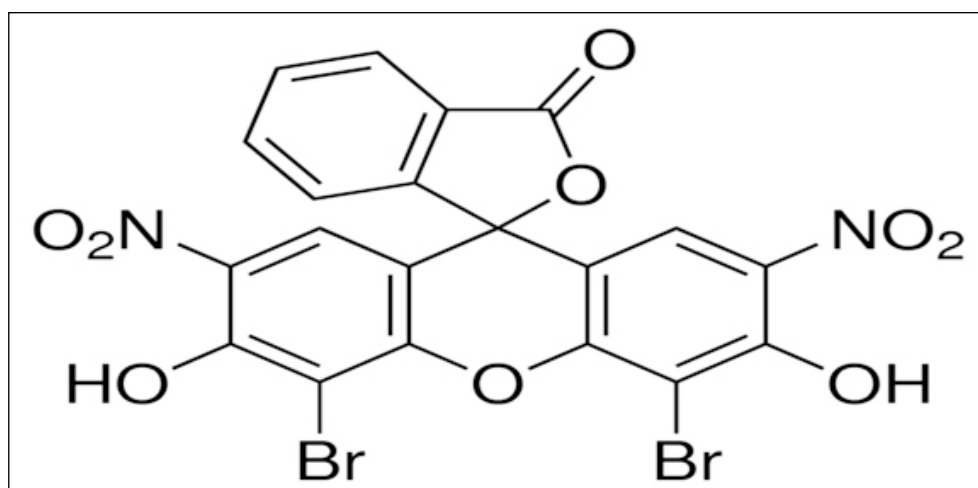**Fig. 1: Chemical structure of polysorbate 80 (Sum of w, x, y, and z is 20)**

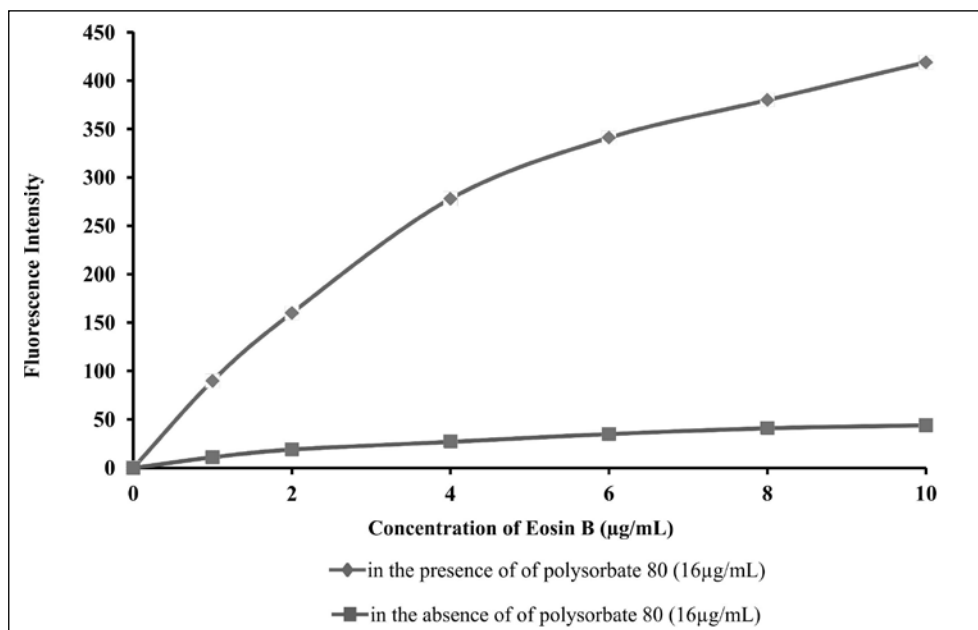
Fig. 2: Chemical structure of eosin B dye

Fig. 3: Influence of Eosin B concentration on fluorescence intensity

Eosin B solution

Eosin B (10 mg) was weighed and transferred to 10 mL volumetric flask, dissolved and diluted with water up to the mark. Aliquot (1 mL) of the resulting solution was transferred to 50 mL volumetric flask and diluted with water up to the mark.

Calibration curve

Appropriate aliquots of working standard solution (4 mg/mL) were taken in a series of 10 mL volumetric flasks. Eosin B solution (2 mL) was added to each volumetric flask and further diluted with citric acid buffer of pH 4.0 to obtain final concentration 16-80 µg/mL of polysorbate 80. The emission spectra of the standard solutions were recorded following excitation at 545 nm. The fluorescence intensities recorded at 580 nm were related to concentration of polysorbate 80. A calibration curve was constructed by plotting fluorescence intensity against corresponding concentration of polysorbate 80, and the regression equation was derived.

Analysis of Sample

The marketed formulation (Lesiq Advance Eye Drops, 0.5% polysorbate 80, 1 mL) was transferred into 10 mL volumetric flask and diluted with water up to the mark (sample solution A). Micro emulsion (10 mL) was prepared in laboratory. Aliquot (1 mL) was transferred to 100 mL volumetric flask and diluted with water up to the mark. Aliquot (1 mL) of resulting solution was transferred to
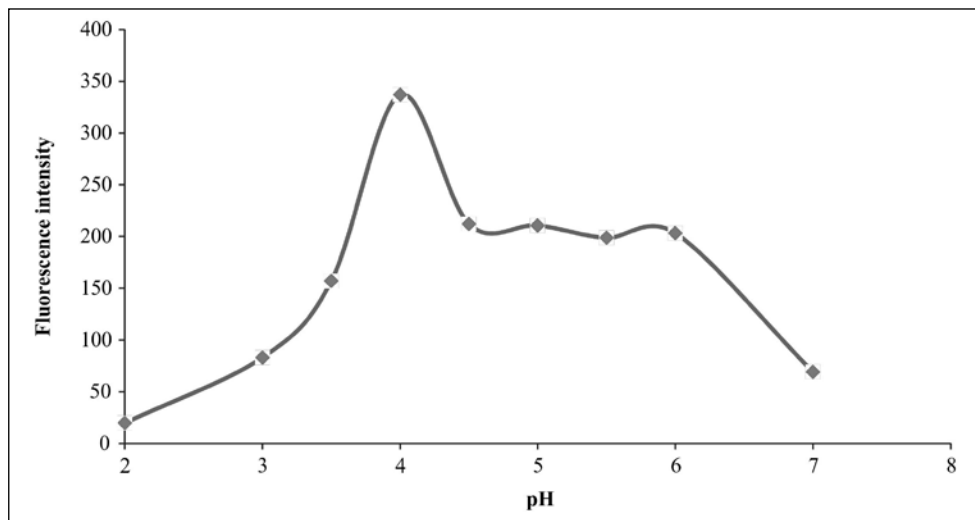
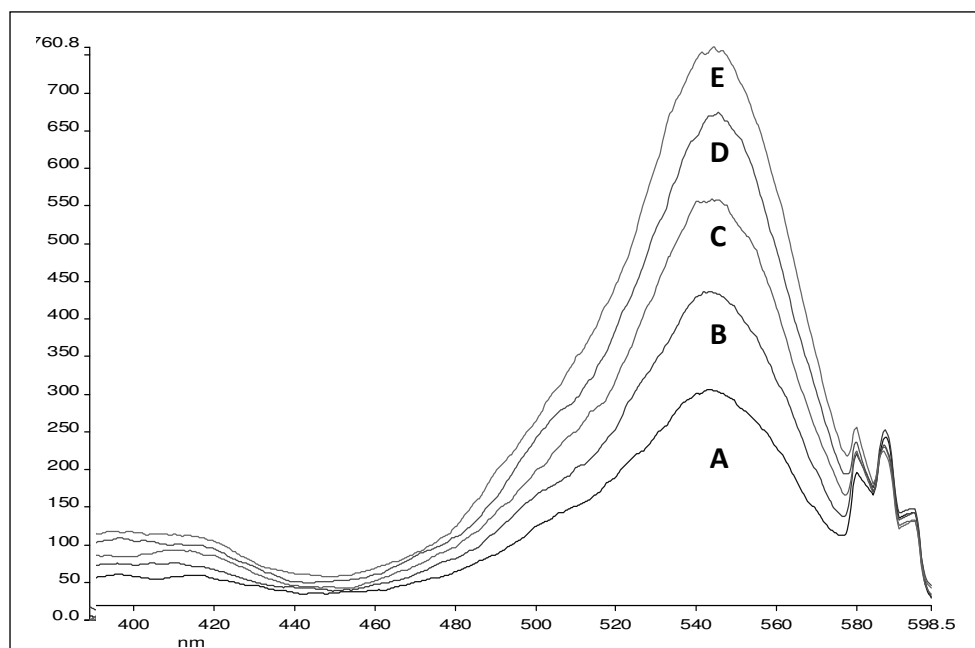**Fig. 4: Influence of pH on fluorescence intensity of Eosin B (4 µg/mL)**

Fig. 5: Influence of concentration of polysorbate 80 (A, 16; B, 32; C, 48; D, 64; E, 80 µg/mL) on fluorescence intensity of Eosin B

METHOD VALIDATION

The method was validated for linearity, precision, accuracy, robustness, selectivity, limit of detection and limit of quantification.

The linearity was studied by preparing standard solutions at different concentration of polysorbate 80 in the range of 16-80 µg/mL. The linearity was assessed in terms of slope, intercept and correlation coefficient for polysorbate 80. The precision of the proposed method was assessed by determination of intra- and inter- day precisions. The intraday precision (% RSD) was determined by analyzing standard solutions of polysorbate 80 (16-80 µg/mL) three times in a day whereas inter-day precision (% RSD) was determined by analysis of polysorbate 80 solutions on three different days. Accuracy of the method was confirmed by recovery study from marketed formulation at three levels of standard addition (50%, 100% and 150%) of label claim in triplicate. The known amount of standard was added to the pre-analyzed sample solution. The limit of detection and limit of quantitation were estimated by serial dilution method.

A series of standard solutions of polysorbate 80 were prepared in the range of 2-32 µg/mL and fluorescence intensity was found. The selectivity of the method was evaluated by analyzing polysorbate 80 in presence of excipients that might be encountered in pharmaceutical formulations.

To evaluate robustness of the method, a few parameters were deliberately varied. The parameters included variation of change in pH of buffer and wavelength. Robustness study was carried out using change in pH of citric acid buffer (3.8 and 4.2) and change

10 mL volumetric flask and diluted with water up to the mark (sample solution B). Aliquot (1 mL) of the sample solution A or B was transferred to 10 mL volumetric flask containing 2 mL of eosin B solution. The volume was made up to the mark with citric acid buffer solution (pH 4.0). The fluorescence intensity of the final sample solution was determined using excitation wavelength at 545 nm and emission wavelength at 580 nm. The amount of polysorbate 80 was calculated using linear regression equation $y=7.54x+110.1$, where y is fluorescence intensity and x is concentration of polysorbate 80.

in excitation wavelength (543 nm and 547 nm). Each factor was studied at 2 levels. The selection of factors and their condition was based on observations during method development and own experience. Experiment was carried out according to above set parameters. Intensity was calculated for polysorbate 80 at each experimental design for spectrofluorimetric method. Standard solution of polysorbate 80 (16 µg/mL) was analysed at each level. The experiment was repeated three times at same condition.

RESULTS AND DISCUSSION

Eosin B shows weak fluorescence because of presence of internal heavy bromine atoms and electron withdrawing nitro groups in molecule. Bromine atoms in the aromatic nucleus generally enhance the intersystem crossing and thus promoting phosphorescence over fluorescence. When non-ionic surfactant such as polysorbate 80 is added to the eosin dye, the fluorescence emission increases along with increase in the concentration of polysorbate 80[16]. The enhancement of the fluorescence intensity of the eosin dye is probably due to the adsorption of dye on the surface of the polysorbate 80. This may further result in an increase of the micro-viscosity of the medium in the vicinity of the dye and hence the fluorescence intensity increases. To develop a new spectrofluorimetric method for analysis of polysorbate 80, different parameters enhancing fluorescence intensity were tested and optimized.

OPTIMIZATION OF EXPERIMENTAL CONDITIONS

Influence of the eosin B concentration

The eosin B concentration was optimized recording fluorescence intensity at different dye concentrations (1-8 µg/mL) in presence and absence of polysorbate 80 (16 µg/mL). The results of these experiments are depicted in Fig. 3. Eosin B showed very weak fluorescence intensity in the absence of polysorbate 80. However, in the presence of polysorbate 80, the fluorescence intensity of eosin B was enhanced. There was a steep rise in the fluorescence intensity up to 4 µg/mL of eosin B followed by a gradual increase at higher concentrations.

Influence of the pH

The effect of pH on the fluorescence intensity was evaluated by diluting mixture of eosin B (4 µg/mL) and polysorbate 80 (16 µg/mL) with citric acid buffer solutions of varying pH. It was found that maximum fluorescence intensity was achieved at pH 4.0 (Fig. 4). Therefore, citric acid buffer with pH 4.0 was chosen as the optimum pH for measurements.

Influence of the polysorbate 80 concentration

The influence of polysorbate 80 concentration was studied using increasing concentrations of polysorbate 80 (16-80 µg/mL) in presence of eosin B (4 µg/mL) at pH 4.0. It was observed that as the concentration of polysorbate 80 increases, the fluorescence intensity increases (Fig. 5).

Method Validation

The validation of the proposed method was performed using the above-mentioned optimum conditions. The proposed method was validated as per the ICH guideline. The validation parameters include linearity, limits of detection and quantitation, accuracy, precision and robustness.

The calibration graph plotted by relating the fluorescence intensity against corresponding concentration of polysorbate 80 was linear over the concentration range of 16-80 µg/mL. High linearity was indicated by the high value of the correlation coefficient ($r = 0.9990$). The linear regression equation was found to be

$y=7.54x + 110.1$

where, y is fluorescence intensity and x is concentration of polysorbate 80.

The limits of detection (LOD) and quantitation (LOQ) were found to be 2 µg/mL and 16 µg/mL, respectively. The intra- and inter-day precision (% RSD) for the determination of polysorbate 80 using proposed spectrofluorimetric method was found to be in the range of 1.04-2.75 % and 1.07-2.90 %, respectively (Table I). The % recovery was found to be in the range of 99.05-99.16 (Table II). The method was found to be robust against deliberate changes made in the pH and detection wavelength.

The laboratory prepared pharmaceutical formulation was analyzed for polysorbate 80 and values of % recovery and % RSD indicated the absence of interference from the commonly found excipients in determination of polysorbate 80.

Pharmaceutical Analysis

The proposed method was successfully applied to the determination of polysorbate 80 in laboratory-prepared micro-emulsion formulation and marketed pharmaceutical formulation. The obtained mean % recovery ranged from 98.14-101.06, indicating the ability of the method to determine polysorbate 80 in pharmaceutical formulations without any interference from co-formulation excipients (Table III).

CONCLUSION

The proposed spectrofluorimetric method is based on the enhancement effect of polysorbate 80 on the native fluorescence of eosin B dye. This method is simple, rapid and sensitive compared to the more sophisticated chromatographic methods. The proposed method is readily applicable for determination of polysorbate 80 in pharmaceutical formulations.

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