Permeability of Piroxicam with Sodium Lauryl Sulfate as Surfactant

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Abstract. Piroxicam is a nonsteroidal anti-inflammatory drug (NSAID) which have characteristic insoluble in water and poorly fluidity. The profile absorption from piroxicam was depend on its dissolution rate in the gastrointestinal tract (GI). To improve the solubility in the water, it can used Sodium Lauryl Sulfate (SLS) as a surfactant which have mechanism by forming a micelle. The aim of this study was to measure the influence gradient concentration of SLS with Piroxicam absorption that can observe by inverted intestinal sac method. The concentration of piroxicam solution that added by SLS are 1, 2, and 3% respectively. The preparation method using Crane and Wilson tube containing mucosal fluid. After preparation, it was taken at 37°C in waterbath and added with serosal solution to intestinal sac by turned upside down and tied to a cannula. The sample was placed in the tube containing mucosal fluid and constantly flowing by oxygen gas. To measure the ab sorption of piroxicam that used serosal solution which every 15 minutes was taken on tube. After this, it was diluted using Ba(OH)2 and ZnSO4 then continuous with separating process. The absorbant of supernatant was measured using Spectrophotometer UV-Vis. Data analyse was calculated by one-way ANOVA. According to Pmax, the values 0.25x10⁻⁵ cm/minute (0% SLS), 0.39x10⁻³ cm/minute (1% SLS), 1.45x10⁻³ cm/minute (2% SLS) and 1.26x10⁻³ cm/minute (3% SLS) respectively. In conclusion, 2% SLS significantly give the highest absorption of piroxicam by altering the membrane permeability.

1. Introduction
There are several factors that can affect the absorption process, one of them is drug solubility. Drug which have low solubility in water, dissolution rate is the rate limiting step of drug bioavailability [1]. Absorption of a drug can be defined as a process of transferring drug through biological barrier into the blood and lymphatics system. Drug absorption can determined by in vitro, in situ and in vivo method. Absorption in vitro through the intestine is based for determining the cumulative absorption of drug from intestinal lumen. This method is used for study various influential factors against permeability of the intestinal wall. Development for design of drugs to optimize the bioavailability especially for the drug with poor solubility and very slow absorption rate [2].

Physicochemical profile of drug is important to determine the bioavailability profile. It is not only important to optimize the formulation but also it can used to evaluation of medicinal products [3]. Piroxicam is a crystalline powder colorless, odorless, bitter taste, in the form of yellow monohydrate. Insoluble in water, in dilute acids and cyclohexane, slightly soluble in methanol, ethanol, isopropanol,
in dimethyl formamide (1:10), dimethylsulfoxide (1:50), acetone (1:50), ethyl acetate (1:80), chloroform (1:20), and solubility in alkaline solution (1:100) (Florey, 1986). The principle of piroxicam solubility is stable at pH 7.5 with pKa 6.3. Factors that influence absorption rate i.e. pH, buffer, temperature and the presence of additional ingredients such as surfactant [4]. Sodium lauryl sulfate (SLS) can reduce surface tension between drug and medium at once form micelles so the drug molecules will carried by the micelle dissolves into the medium [5]. Addition of surfactant was assumed to be able to interact complex with drugs then affect the permeability of the membrane [6]. The permeability is affected by the diffusion coefficient, surface area, partition coefficient and a thick membrane.

Sodium Lauryl Sulfate (SLS) is anionic surfactant, stable in many medium, easy to mix with other components, non-toxic, no irritating, and effective in a wide pH range [7]. One of the important thing is the ability of surfactant to improve drug solubilization which insoluble or small dissolved in a medium. Surfactant on low concentration, reduce surface tension and increase the absorption rate of drugs [8]. Surfactant in high concentration will gather to form aggregates called micelles [9].

2. Experimental

2.1. Materials and Instrument
Piroxicam (Nantong General Pharmaceutical Factory), SLS (Merck), NaH₂PO₄ (Merck), Na₂HPO₄ (Merck), NaOH (Merck), Ba(OH)₂ dan ZnSO₄ (Merck), dan NaCl (Merck), aquadesitlata and male Wistar rat. Instrument which were used in this research are Crane and Wilson tube were modified by Yuwono from Institut Teknologi Bandung (ITB), Spectrophotometer Genesys 10, Analytical balance (Shimadzu Scientific Ltd.), Digital pH meter Omega models 5003, Centrifuges MLW T51.1, Sonicator Elma T 570, Waterbath (Shimadzu Scientific Ltd.), tools for surgery, glassware, volume pipette, test tube and flask.

2.2. Procedure

2.2.1. Calibration Curve of Piroxicam. Stock solution was prepared from 100 mg of Piroxicam were dissolved in a sonicator and then added with phosphate buffer (pH 7.4). The Series of stock solution i.e 10, 12, 14, 16, 18 μg/ml, then takes 1.0 ml of each series add 4.0 ml SLS (1% w/v) in phosphate buffer solution pH 7.4. The solution was measured at a maximum absorbance wavelength (200 -400 nm).

2.2.2. Permeability Test. Male wistar rats are adapted in the experimental room for a week before test. Male wistar rats are observed health and behavior. Male wistar rats which used in the experiment were healthy, showed no behavioral abnormalities and deviations from normal circumstances, and no weight loss exceeded 10% [10]. Principles of laboratory animal care guidelines were followed and prior permission for conducting the study (Ref. No. FO-UGM-FA-13-03).

Determination of the membrane permeability was observed with intestinal inverse sac method. Pylorus portion from male wistar was removed and then collected the intestine part. It was cleaned with NaCl 0.9% w/v and then divided into 2 equal length. In this study was using the top part intestine to placed sample treatment (0%, 1%, 2% and 3% SLS) and the bottom part as a negative control (without piroxicam).

Mucosal solutions are made from 20 mg% piroxicam in phosphate buffer pH 7.4 with SLS at level 0, 1, 2, and 3% (w / v). Mucosal solution put in a waterbath at a temperature of 37°C. 1.5 ml of serosal solution incorporated into the intestinal sac that has been turned upside down and tied to the cannula, then inserted into the tube which already contains the mucosal fluid and constantly flowing O₂ gas at a rate of approximately 100 bubbles per minute to maintain the structure of the intestinal wall. During the trial maintained that all parts of the intestinal mucosal submerged in the liquid. Samples were taken every 15 minutes until 75th minutes.
Serosal fluid’s sample was taken from intestinal sac then added with ZnSO₄ 5% and 0.3 N Ba(OH)₂ than centrifuged for 25 minutes. Supernatant was taken in tube and measured in the maximum wavelength of absorbance by spectrophotometre UV-Vis.

Total cumulative piroxicam was calculated from calibration curve of piroxicam, absorbance of sample reduced with negative control. Absorption rate, permeability of membrane, and lag time were calculated from the regression equation curve cumulative amount of piroxicam absorbed after steady state conditions in each replication. The data were analyzed with one way ANOVA test to compare the value of absorption rate, permeability of membrane and lag time between any various concentration of surfactant.

3. Result and Discussions

3.1. Calibration Curve of Piroxicam

![Figure 1. Wavelenght Maximum of Piroxicam.](image1.png)

![Figure 2. Calibration Curve of Piroxicam.](image2.png)
Wavelength maximum of Piroxicam was 356 nm. Drug absorption from gastrointestinal tract into the blood generally occurs after it dissolved in the absorption site. Solubility related with absorption rate which has a linear curve. SLS as a material that can increase the solubility of drug and it was expected to increase the piroxicam absorption rate.

3.2 Permeability Test

Table 1. Total cumulative piroxicam was absorbed (x 10^-2 mg).

| Time (minute) | SLS 0% | SLS 1% | SLS 2% | SLS 3% |
|---------------|--------|--------|--------|--------|
| 15            | 0      | 0.540±0.431 | 2.268±1.773 | 1.512±1.292 |
| 30            | 0.144±0.125 | 1.320±1.238 | 5.655±3.589 | 4.998±3.434 |
| 45            | 0.548±0.250 | 2.355±1.003 | 9.579±4.179 | 8.232±4.106 |
| 60            | 1.469±0.173 | 3.243±1.222 | 14.086±6.661 | 12.983±6.071 |
| 75            | 2.358±0.350 | 5.156±1.894 | 19.727±8.324 | 16.165±8.105 |

Table 2. The value of absorption rate (K), permeability of membrane (Pm), and the lag time in various concentration of SLS.

| Groups       | Absorption rate (μg/minute) | Permeability of membrane (x10^-3 cm/minute) | Lag time (minutes) |
|--------------|-----------------------------|---------------------------------------------|--------------------|
| SLS 0%       | 0.050±0.009                 | 0.250±0.043                                 | 29.480±4.400       |
| SLS 1%       | 0.077±0.017                 | 0.390±0.087                                 | 13.874±10.474      |
| SLS 2%       | 0.289±0.107                 | 1.440±0.538                                 | 10.309±3.407       |
| SLS 3%       | 0.252±0.103                 | 1.260±0.516                                 | 12.054±6.416       |

The value of Absorption rate, permeability of the membrane, and the lag time was linier with total cumulative piroxicam curve after steady state conditions. Table II showed the absorption rate, permeability of membrane, and lag time related with increased levels of SLS. The results showed that 2% SLS give the highest absorption. SLS which have highly polarity was easily soluble in water. Interaction between piroxicam-SLS formed a complex which is more soluble in water and intestinal fluid compared with piroxicam.

4. Conclusions
The highest absorption in solution piroxicam is addition 2% SLS and then followed by addition 3%, 1% and 0% SLS seen from the Pm, which is 1.44; 1.26; 0.39; and 0.25 (x10^-3 cm / minute).

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