Abstract
Linagliptin is a highly selective inhibitor of dipeptidyl peptidase-4 and therefore clinically utilized to treat adults with type 2 diabetes mellitus. The objective of this study was to predict how the ions normally found in extracellular or intracellular fluids within the body system will influence the linagliptin’s rate of passive diffusion across a cell membrane. The methodology involved measuring linagliptin partition coefficient in a chloroform-water system containing the salts at 25 °C by the shake flask method. The results indicate that at the highest concentration (0.5 M) of the salts studied, sodium chloride was found to give the highest partition coefficient value when compared to the control. In conclusion, physiological ions would have little or no effect on the drug's molecular state within extracellular or intracellular fluid as the salts failed to significantly alter the partition coefficient of the drug, hence will not alter passive membrane permeability of linagliptin.

Keywords: Linagliptin, partition coefficient, salts (electrolytes), passive diffusion.
pressure (sodium, chloride ions); enzymatic activities (magnesium ions); blood clotting and bone integrity (calcium ions) are found both in extracellular and intracellular fluids. The rate of passive diffusion across a cell membrane has been reported to be proportional to the partition coefficient of the drug between the external medium (aqueous environment) and the lipophilic cell membrane; the diffusion coefficient of the drug through the membrane, and the drug’s concentration gradient across the membrane [6]. The membrane permeability controls the uptake and efflux of drugs in relevant compartments. To predict passive membrane permeability, lipophilicity is the most critical parameter to be investigated [7]. The logarithm partition coefficient (log P) of a chemical substance between an aqueous and organic phase, (usually water and octanol) is the parameter which determines the lipophilicity of a chemical substance [8]. Since most drugs pass at least one cellular membrane to reach the site of action, it becomes important to know how electrolytes present in biological fluids compartments affect the lipophilicities of active chemical substance (drug) molecules. Linagliptin was the drug of choice in the present study amongst DPP-4 inhibitors, because it is the only drug in this class that most of the absorbed drug is excreted unchanged through non-renal route.

Thus, in an attempt to verify if biological fluid ions affect the lipophilicity of linagliptin and hence its excretion, the present study, investigated the influence of electrolytes (salts) on the partition coefficient of linagliptin.

**Experimental**

UV/Vis spectrophotometer (Jenway 6305, England), linagliptin (Sreeven Pharma Pvt, India), the salts (sodium chloride, sodium sulfate, potassium chloride, calcium chloride, cobalt chloride, potassium sulfate, magnesium sulfate, aluminum sulfate) were purchased from Fischer Scientific, (USA). All other chemicals were of analytical grade and double distilled water was utilized in the analysis.

**General procedures**

The standard solution of linagliptin was prepared by accurately weighing 10 mg of the reference drug and transferred to a 100 ml volumetric flask, dissolved in methanol and diluted to volume with methanol (stock solution A). Stock B flasks solution was prepared by diluting 1 ml of stock A to 10 ml with methanol in a volumetric flask. Working standard solutions (1.0, 2.0, 3.0, 4.0, 5.0 µg/ml) were prepared in volumetric from stock B solution.

The salt solutions were obtained by preparing a stock of one molar (1M) solution of each salt in double distilled water. Working salt solutions (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 M) were prepared by diluting the stock solution.

Linagliptin partition coefficient was measured in a chloroform-water system. To 5 ml of saturated chloroform in a vial containing 50 µg/ml of linagliptin was added 4 ml of distilled water (control) or saturated aqueous solution of each of the salts studied. The vials were capped and agitated at room temperature for 2 h to obtain complete equilibration. The mixture was transferred to a separating funnel and the phases were allowed to equilibrate for about 15 min and separate. The aqueous phase was analyzed at a maximum wavelength of 292 nm using UV/Vis spectrophotometer. Linagliptin concentration was obtained from a pre-constructed calibration graph. The partition coefficient of the drug was calculated using equation 1 [8].

$$P = [C_r - C_0]V_A/V_O, \quad \text{equation 1}$$

Where $P$ is the partition coefficient; $C_r$ is the total concentration of linagliptin; $C_0$ is the concentration of linagliptin in aqueous phase; $V_A$ is the volume of the aqueous phase; $V_O$ is the volume of organic phase.

**Results and Discussion**

A plot of absorbance versus concentration, gave a linear graph. The regression analysis of the plot gave an equation: $\text{Abs} = 0.05996C - 0.00762$. The linearity (defined by the correlation coefficient of 0.9992) of the plot between absorbance and concentrations of the drug shows that Beer-Lambert law was obeyed.

The results obtained from the partition coefficient analysis are presented in Table 1.

**Table 1: Partition coefficient of linagliptin**
The results indicate that the partition coefficient of linagliptin increased as the concentrations of the electrolytes (salts) were being increased. At the highest concentration level (0.5 molar) studied, cobalt (Co³⁺) ion produced the least partition coefficient value of the drug. With the chloride anion (Cl⁻), it was observed the order of increase in the partition coefficient of linagliptin is Na⁺>K⁺>Ca²⁺>Co³⁺. Similarly with sulfate anion (SO₄²⁻), it was also noted that the order of increase in the partition coefficient of linagliptin is Na⁺>K⁺>Mg²⁺>Al³⁺. However, with the same cation (for example Na⁺ ion), the order of increase in the partition coefficient of the drug is Cl⁻>SO₄²⁻. Partition coefficient is extensively utilized to predict the bioactivity of drugs as it exhibits interesting superficial similarity with lipids [10].

The correlation between experimental partition coefficient and the concentration of the salts was expressed by plotting logarithm partition coefficient of the drug versus concentration of salts (Figures 2 and 3 respectively) and linear relationships were observed. The correlation coefficients of these linear plots are 0.9612, 0.8811, 0.9048, 0.9771, 0.9831, 0.9951 for sodium chloride, potassium chloride, calcium chloride, sodium sulfate, potassium sulfate and magnesium sulfate respectively. Furthermore, the correlation coefficients (plots not presented) of cobalt chloride and aluminum chloride were found to be 0.9949 and 0.9859 respectively.

Conclusion
The partition coefficient values of linagliptin were not significantly altered by the salts (electrolytes) investigated, implying that physiological ions would have little or no effect on the drug’s molecular state in either the extracellular fluid or intracellular fluid, and therefore will have no effect on passive membrane permeability of linagliptin. Furthermore, the study reveals that linagliptin can be concomitantly administered with inorganic drugs like antacids or antiemetics, without the therapeutic effect of linagliptin being altered.
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