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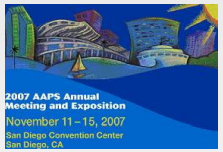

EFFECT OF IONIZATION AND LIPOPHILICITY ON PERMEATION OF MODEL MOLECULES THROUGH URINARY BLADDER EPITHELIUM

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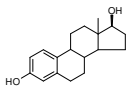
INTRODUCTION

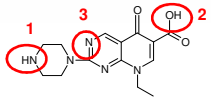
The main function of the urinary bladder is to act as a short-term storage site for urine, while maintaining the composition of the urine similar to that generated by the kidneys. With the exception of actively transported substances, the urothelium should be impermeable to all substances present in urine or blood. However, there are experimental data that suggest drug reabsorption from bladder. This could influence the pharmacokinetic profile of drugs eliminated by renal excretion and also the efficacy, for example, of citotoxic drugs commonly used in urological practice (intravescical instillation) for treatment of superficial bladder cancer.

OBJECTIVES

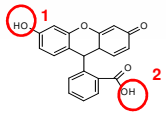
The aim of this work was to characterize the permeation properties of model molecules across pig urinary bladder epithelium in vitro. In particular, the effect of lipophilicity of the permeant on epithelial permeability was studied using molecules with similar molecular weight (estradiol, pipemidic acid and fluorescein) but different lipophilicity. Moreover, we explored the effect of electric charge of model molecules (pipemidic acid and fluorescein) on their epithelial permeability.



17-β ESTRADIOL



PIPEMIDIC ACID



SODIUM FLUORESCEIN

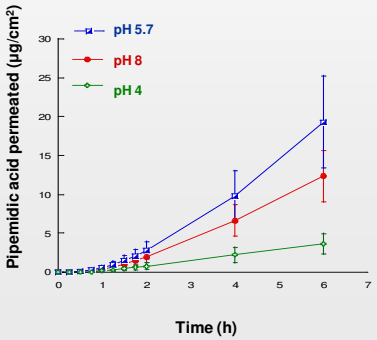
	17-β ESTRADIOL	PIPEMIDIC ACID	SODIUM FLUORESCEIN
MW	272.4	303.3	376.3
pKa	pKa: >8.5	pKa (1) : 7.98 ± 0.25 pKa (2) : 4.11 ± 0.20 pKa (3) : 1.40 ± 0.20	pKa (1): 6.8 pKa(2):4.45
	Log P: 4.01	Log D: -2.13 (pH 4) -1.75 (pH 8) -1.58 (pH 5.7)	Log P: -1.52

Calculated electrical charges (Handerson-Hasselbach equation):

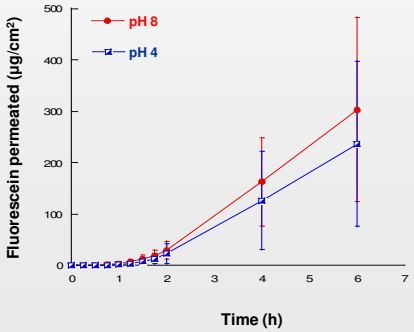
$$pH = pKa + Log\frac{A^-}{AH}$$

pH	Pipemidic acid	Sodium fluorescein
4	+ 0.5	-0.5
8	-0.5	-2
5.7	neutral	Not tested

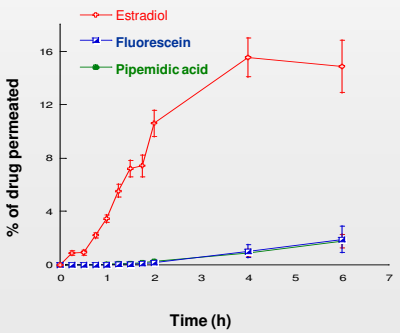
Pipemidic acid: effect of ionization



Fluorescein: effect of ionization



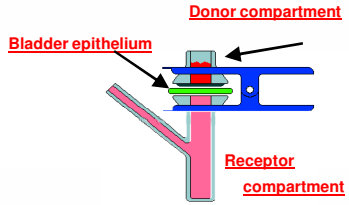
Effect of lipophilicity



METHODOLOGY

Evaluation of Trans-Epithelial Resistance (TER)

- Careful dissection of the smooth muscle layer with a scalpel, in PBS at 4°C.
- Bladder epithelium mounted on a Franz-Type diffusion cell.
- Two electrodes (Ag/AgCl) placed in the donor and receptor compartment, both filled with PBS, pH 7.4.
- Measurement of TER with a digital multimeter.
- Discard specimen with initial resistance lower than 1000 Ω/cm^2.



Permeation experiments

- Performed for 6 hours at 37°C in Franz-Type diffusion cells, under constant stirring.
- Donor solutions of model drugs prepared in PBS at different pH.
- At the end of the permeation experiments TER were evaluated.
- Samples were analyzed with HPLC with UV-Visible or fluorimetric detection.

CONCLUSIONS

✓ Pipemidic acid at pH 4 is positively charged and it shows lower permeation profiles through the bladder epithelium: this could be due to the presence of glycosaminoglycans on the bladder epithelium surface, which are positively charged.

✓ The quantity of charge doesn't affect the permeation profiles (see fluorescein at pH 4 and at pH 8).

✓ Estradiol, the molecule with the highest lipophilicity, has a higher permeation profile through the bladder epithelium.