RESEARCH ARTICLE

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Preparation and Physicochemical Characterization of Mosapride Citrate Pharmacosomes

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Abstract: The objective of this research was to improve the physicochemical properties of mosapride citrate (MSP) with an ultimate goal to increase its bioavailability. In order to achieve this goal, MSP-pharmacosomes were prepared by refluxing MSP and phospholipids at different temperatures, for different times and using different mosapride: phosphatidylcholine (PC) molar ratios. The yield % of prepared MSP-pharmacosomes was evaluated to determine the best preparation conditions. The best formula was characterized regarding N-octanol/water partition coefficient, N-octanol solubility; water solubility, FT-IR, differential scanning calorimetry (DSC), X-ray diffraction (XRD) morphology and particle size. Results revealed that reaction temperature 60°C for reaction time of 2 hrs using MSP: PC molar ratio 1:2 was the optimum conditions for MSP-pharmacosomes preparations. The prepared MSP-pharmacosmes partition coefficient was higher than MSP material. MSP-pharmacosomal dispersion in water had a small particle size in nano range. Accordingly, MSP-pharmacosomes could be a promising approach for improving physicochemical properties of mosapride citrate to increase its bioavailability.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is the most common gastrointestinal diagnosis recorded during visits to the clinics. [1] GERD is defined as "a condition which develops when the reflux of stomach contents causes troublesome symptoms and/or complications". [2] The main problem in GERD is that the exposure of esophageal epithelium to gastric secretions may lead to a histopathologic injury or symptom elicitation. [3]

Mosapride citrate is (4-Amino-5- chloro-2-ethoxy-N-((4-(4-fluorobenzyl)-2-morpholinyl) methyl) benzamide (citrate dihydrate) Figure 1. [4] Mosapride citrate (MSP) is a selective serotonin 5-HT4 receptor agonist drug used for short-term treatment and relieving symptoms and endoscopic relapse in patients with mild GERD. [5-8] MSP is used also for gastrointestinal symptoms associated with chronic gastritis, irritable bowel syndrome and functional dyspepsia. [8] MSP oral bioavailability is about 8% which is low. [9] Changing the drug physicochemical properties may increase its bioavailability. In this study, changing MSP physicochemical properties was achieved by preparation of pharmacosomes.

Pharmacosomes can be defined as "colloidal dispersion of drugs covalently linked to lipids". [10] Pharmacosomes are stoichiometric complexes of polyphenolic compounds with phospholipids, mainly phosphatidylcholine (PC). [11] For the drug to be esterified to the lipid, it must have an active hydrogen atom (-COOH, -OH, -NH2 etc.). [11] In aqueous colloidal dispersions, pharmacosomes consist of vesicular or micellar formations. [12]

The main difference between pharmacosmes and liposomes is that liposomes are prepared by drug Incorporation in the aqueous or lipid phase of a mixture of lipids while pharmacosomes are prepared by Covalent binding of a drug to a lipid where the resulting complex is the carrier and the active compound at the same time. [11] As the drug molecules are covalently bound to lipids, pharmacosomes avoid the usual problems associated with liposomes like entrapment of polar drug molecules, low drug incorporation, leakage and stability problems. [10]

The aim of this work is to improve the physicochemical properties of mosapride citrate (MSP) with an ultimate goal to increase its bioavailability. This is done through the preparation of mosapride citrate pharmacosomes (MSP-pharmacosmes). During the study, the best conditions for preparation of MSP-pharmacosmes were evaluated followed by physicochemical characterization of formed MSP-pharmacosomes.

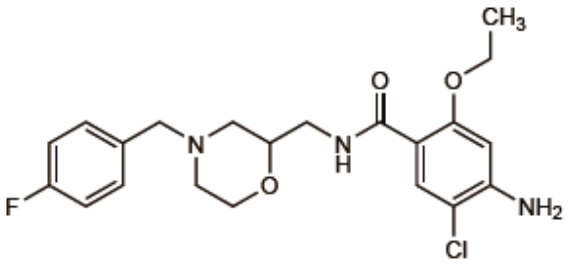

Figure 1: Mosapride

MATERIALS AND METHODS

Mosapride citrate (a kind give from Marcyrl Company), soya bean phosphatidylcholine and tetrahydrofuran were obtained from sigma-aldrich, st Louis, USA. Methanol and glacial acetic acid were purchased from Adwic, El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

Preparation of Mosapride Citrate Pharmacosomes

There are various methods for pharmacosomes preparation to form a stable covalent bond between drug and phospholipids. Basically, a drug with an active hydrogen group is reacted with phospholipids in the presence of a suitable organic solvent to form pharmacosomes. [11]

MSP pharmacosomes were prepared by refluxing Weighed amount of MSD and phospholipids in a 100 ml round bottom flask containing tetrahydrofuran as reaction solvent at the specified temperature for the specified time. After then the tetrahydrofuran was evaporated off under vacuum at 40°C, the dried residues were collected and placed in desiccators overnight. [13]

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Effect of Reaction Temperature

The reaction temperature for MSP-pharmacosmes preparation was controlled at 40, 50 and 60°C using MSP: PC ratio 1:1 and reaction time of 1 hour.

Effect of Reaction Time

A reaction time of 1 and 2 hrs were used to determine the effect of time on MSP-pharmacosomes preparations. The reaction temperature was maintained at 60°C using MSP: PC ratio 1:1.

Effect Ratio of Mosapride Citrate and Phospholipid

MSP-pharmacosomes were prepared using ratios of MSP: soybean lecithin 1:1 and 1:2. The reaction time was maintained at temperature 60°C for a reaction time of 2 hours.

The Yield of MSP-Pharmacosmes (%)

The MSP-pharmacosmes prepared as above were dissolved in methanol to determine total MSP content in MSP-pharmacosomes spectrophotometrically at λ_{max} 308 nm. To determine un-reacted MSP, the prepared MSP-pharmacosmes were washed by dispersion in 1% acetic acid to dissolve un-reacted MSP. The dispersion is then filtered and un-reacted MSP dissolved in 1% acetic acid was determined spectrophotometrically at λ_{max} 308 nm.

$$\text{The yield} = \frac{a - b}{a} x\ 100$$

Where a is total MSP and *b* is free un-reacted MSP.

Further Characterization of Selected MSP-Pharmacosomes

1. N-octanol/Water Partition Coefficient (P) of MSP-Pharmacosomes

N-octanol/water partition coefficient determination of MSD material and MSP-pharmacosomes was carried out by adding 0.1 g of MSD material, or MSP-pharmacosomes to 10 ml water in sealed glass containers at 37°C, the liquids were agitated for 24 h and centrifuged to remove excessive residues (15 min, 4000 rpm). 10 ml n-octanol was then added after removing excess residue and agitated for 24 h. Then they were centrifuged at 4000 rpm for 15 min. The water phase and n-octanol phase were separated. The water phase and n-octanol phase were filtrated through a 0.45 µm membrane. The concentration of MSD was measured spectrophotometrically using UV at wave length 309 nm.

$$P = Co/Cw$$

where Co was the concentration of MSD in n-octanol; Cw was the concentration of MSD in water.

2. Solubility Studies

Excess amount of MSP and MSP-pharmacosomes was added to 5 ml water or n-octanol in a sealed glass container at 37°C. The liquids were agitated for 24 h and then centrifuged to remove excessive MSP and MSP pharmacosomes. The obtained solution was filtrated through a 0.45 µm membrane. Then the absorbance of each system was recorded in ultraviolet-visible spectrophotometer.

3. FT-IR, DSC and XRD characterization of MSP Pharmacosomes

FT-IR, DSC and XRD were used to characterize the MSP-pharmacosomes. The FTIR was recorded using a Bruker FTIR spectrophotometer (Model 22; Bruker, Coventry, UK). DSC was performed using a Shimadzu differential scanning calorimeter (DSC-50, Shimadzu, Kyoto, Japan). XRD was conducted using a Scintag diffractometer (XGEN-4000, Scintag Corp., Sunnyvale, CA, USA).

4. Transmission Electron Microscopy

Sample of MSD-pharmacosomes was examined by transmission electron microscope (TEM) operated at 80 kV (model JEM-1230, Jeol, Tokyo, Japan).

5. Particle Size (PS) Analysis

The mean PS was determined by Malvern Zetasizer at 25°C (Malvern Instrument Ltd., Worcestershire, UK). Before PS measurement, MSP-pharmacosmes Formula was properly dispersed in distilled water to form a diluted dispersion with suitable scattering intensity.

RESULTS AND DISCUSSION

Preparation of Mosapride Citrate Pharmacosomes

3 Different temperatures were used in order to investigate the effect of the reaction temperature on yield %. Percent yield for MSP-pharmacosmes prepared at 40°C was 63.46 ± 1.45. The yield % was significantly increased to 74.66±2.51 by increasing reaction temperature to 50°C (p<0.05). Further increase in reaction temperature from 50 to 60 °C lead to a significant increase in yield % to 83.25±1.30 (p<0.05). These results are in agreement with other results published in the literatures. [13, 14]

Regarding the reaction time, it was obvious that increasing the reaction time from 1 to 2 hrs lead to a significant increase in yield % from 83.25±1.30 to 90.90±2.27 (p<0.05). Finally, increasing MSP: PC molar ratio from 1:1 to 1:2 also lead to a significant increase in yield value to 96.06±0.18 (p<0.05). Accordingly, F5 prepared at reaction temperature 60°C for reaction time of 2 hrs using MSP: PC molar ratio 1:2 was chosen as optimum formula for further characterizations.

Further Characterization of Selected MSP-Pharmacosomes (F 5)

1. N-octanol/Water Partition Coefficient (P)

Lipophilicity determined as expressed as N-octanol/water partition coefficient is very important in analysis of absorption related physicochemical properties. [15] It's well known that increasing partition coefficient increases drug permeability into cells. [16] Transcellular drug diffusion depends mainly on the drug lipophilicity as the compound needs a certain affinity to lipid structures for cell membrane entry. [17] Table 2 shows the n-octanol/water

Table 1: The Yield % of Prepared MSP-Pharmacosomes

Formula [a]	EA used in the Formulation			Yield %[a]
	Reaction Temperature (°C)	Reaction Time (hr)	Molar Ratio MSP : PC	
F 1	40	1	1:1	63.46±1.45
F 2	50	1	1:1	74.66±2.51
F 3	60	1	1:1	83.25±1.30
F 4	60	2	1:1	90.90±2.27
F 5	60	2	1:2	96.06±0.18

[a] Each value represents mean ± standard deviation (SD) of three determinations (n = 3)

Table 2: N-octanol/Water Partition Coefficient (P) of MSP and MSP–Pharmacosomes (F5)

	MSP	F5
P	5.74 ± 0.08	49.21 ± 1.94

Table 3: Solubility of MSP and MSP–Pharmacosomes (F5) in Water and N-octanol at 37°C

	MSP	F5
Solubility in water (mg/ml)	1.34 ± 0.12	0.32 ± 0.04
Solubility in n-octanol (mg/ml)	1.87 ± 0.39	4.23 ± 0.14

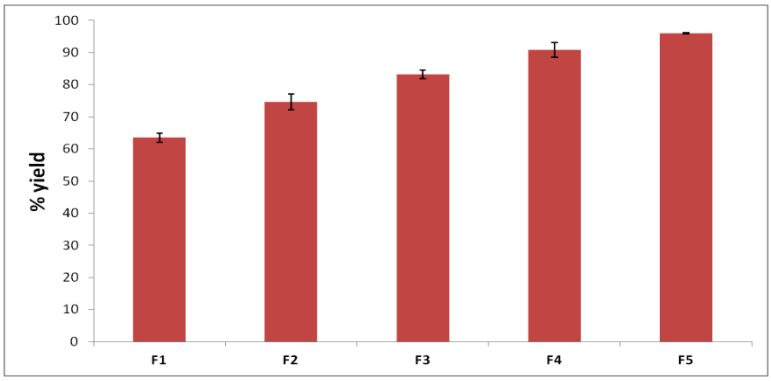

Figure 2: % yield of prepared MSP-pharmacosomes

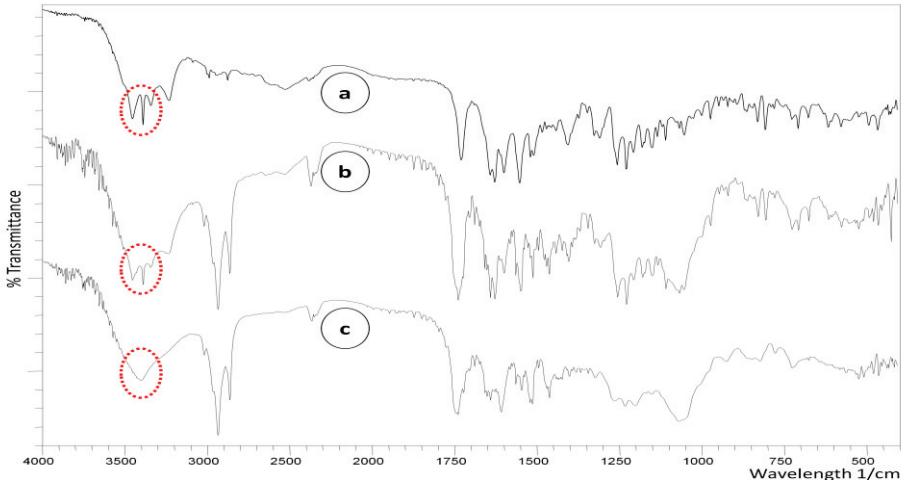

Figure 3: FT-IR spectra of pure MSP (a), MSP and PC physical mixture (b) and the MSP-pharmacosomes F5 (c)

partition coefficient (P) of MSP and MSP–pharmacosomes (F5). The data showed that P of MSP-pharmacosomes in n-octanol and water significantly increased about 10 times more than that of MSP material ($p<0.05$) this increase in lipophilicity may result from due to the strong dispersibility and amorphous form of the MSP-pharmacosomes masking of polar groups of MSP. [13]

2. Solubility Studies

Solubility studies were conducted to check the solubility of MSP after the formation of MSP-pharmacosomes (F5). Table 3 shows the solubility of MSP and MSP-pharmacosomes in water and n-octanol. The data show that pure MSP has slightly more liposolubility than water-solubility. Formation of MSP-pharmacosomes leads to a

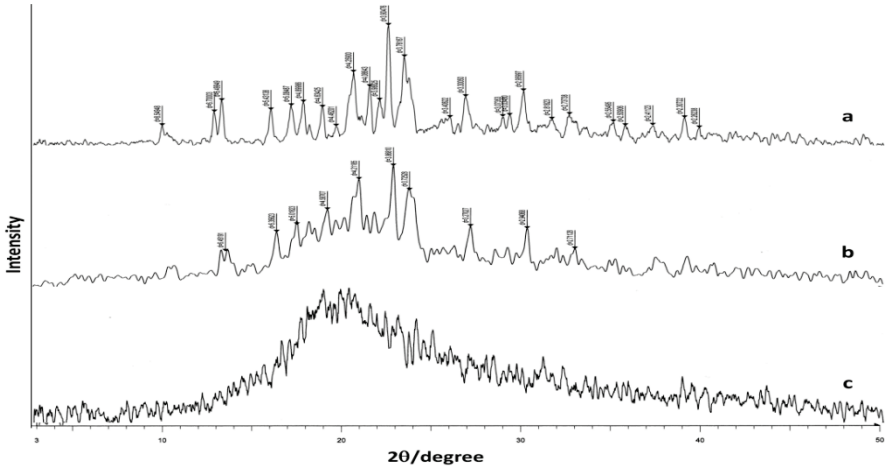

Figure 4: XRD of pure MSP (a), MSP and PC physical mixture (b) and the MSP-pharmacosomes F5 (c)

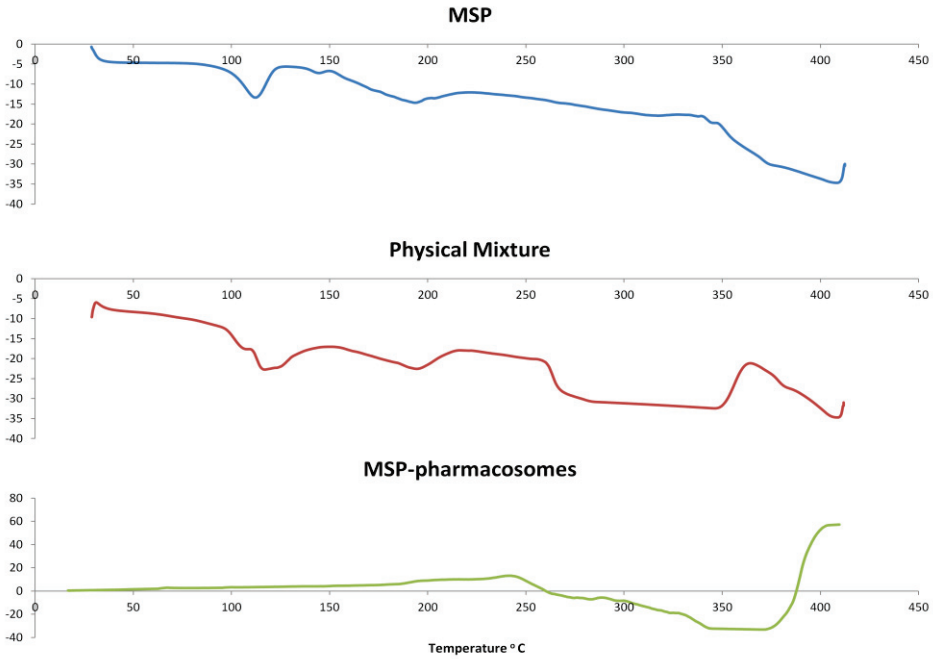

Figure 5: DSC thermograms of pure MSP, MSP and PC physical mixture and the MSP-pharmacosomes F5

significant increase in lipid solubility and significant decrease in water solubility ($p<0.05$). This can explain the marked increase in N-octanol/water partition coefficient of MSP-pharmacosomes.

3. FT-IR, DSC and XRD Characterization of MSP Pharmacosomes

The FT-IR spectra performed for pure MSP, MSP and PC physical mixture and MSP-pharmacosomes F5 shown in Figure 3. By comparing the FT-IR spectra, there was a significant difference between the pure MSP, the physical mixture and MSP-pharmacosomes. The FT-IR spectrum of pure MSP shows two N-H primary stretching vibration bands at 3379 cm^{-1} and 3444 cm^{-1}. In the spectrum of the physical mixture, the characteristic absorption peaks of MSP are still present. However, in the spectrum of MSP-pharmacosomes, the characteristic absorption peaks of MSP are masked and no new peaks were observed. These observations suggest that interactions took place between MSP and PC during the formation of MSP-pharmacosomes. [18]

The powder X-ray diffraction patterns of MSP, physical mixture and MSP-pharmacosomes (F5) is shown in Figure 4. MSP powder diffraction pattern displayed sharp crystalline peaks, which is the characteristic of a crystalline macromolecule. In the physical mixture, crystalline drug signal was still detectable. However, the crystalline peaks had disappeared in MSP-pharmacosomes. This suggested that MSP-pharmacosmes was in amorphous. [19]

Figure 5 illustrates the DSC thermograms of MSP, physical mixture and MSP-pharmacosomes (F5). The thermogram of MSP showed a characteristic endothermic peak at 113°C that corresponds to the melting point of the drug. [20] This characteristic peak could still be detected in the physical mixture thermogram. DSC of phospholipids complex showed this endothermal peak disappeared. This suggests that some interactions between MSP and PC took place.

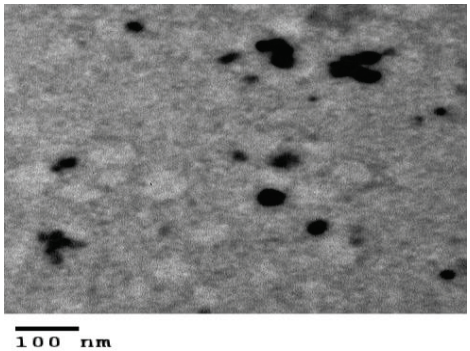

Figure 6: Transmission-electron micrograph of MSP-pharmacosomes F5

4. Transmission Electron Microscopy

The TEM of MSP-pharmacosomes F5after dispersion in distilled water is shown in Figure 6. It can be seen that MSP-pharmacosomes formed a vesicular structure in aqueous dispersions.

5. Particle Size (PS) Analysis

Z-average of the particle size of MSP-pharmacosmes (F5) dispersion in distilled water was in the nanometer range (165.65±16.8 nm) and it was polydispersed as PDI < 1.

CONCLUSIONS

In this research, it has been revealed that the MSP-pharmacosmes are best prepared by maintaining temperature at 60°C for 2 hrs using 1:2 molar ratio of MSP: PC. FT-IR, XRD and DSC results confirmed the formation of MSP-pharmacosomes. The prepared MSP-pharmacosmes exhibited higher partition coefficient and small particle size after dispersion which can increase drug absorption in gastrointestinal tract.

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