Introduction

Aripiprazole (ARP) is an antipsychotic drug with proven therapeutic effect against schizophrenia (1), and approved by the FDA for the treatment of acute manic and mixed episodes associated with bipolar disorder. ARP is a partial D2, and 5-HT1A receptor agonist, and like the other atypical antipsychotics, it is a 5-HT2A receptor agonist (2). ARP is a poorly water-soluble drug and classified as class II drug according to BCS (3). Previous attempts to increase ARP solubility were performed through complexation with cyclodextrins (4, 5), application of nanomilling and coprecipitation (6), formation of nanosuspensions (7), and through formation of 3D printed orodispersible films (8). Incorporation of ARP into nanoparticles like solid lipid nanoparticles (9) and into poly (caprolactone) nanoparticles (10) has also been evaluated.

In the field of pharmaceutical technology, a drug carrier system should be simple, easily prepared, having high entrapment efficiency and a controlled drug release in the body. Recently, silica particles have been exploited as matrices for enhancing the entrapment and dissolution rate of candidate active moieties. Silica particles present an attractive host to the active agent owing to its low toxicity, excellent biocompatibility, particle morphology, and chemical character of the silica surface (11). In 2011, nonporous silica nanoparticles for cancer imaging were approved for the first in-human clinical trial (12,13). Therefore, the polymer-silica composites represents a promising class of materials for drug delivery.

Drug loading within silica carrier can be carried out using various novel loading approaches, viz, microwave irradiation, solvent methods, and supercritical fluid (14-17). Drug molecules can be stabilized either on the silica surface or within silica pores in an amorphous state, which enhances the dissolution rate of...
the drug (18,19). Yilmaz and Bengisu (20) studied the potential
to drug loading onto silica microspheres through a single step
sol–gel process (20).
In this study, tetraethyl orthosilicate (TEOS) has been used as
a carrier for the preparation of ARP-loaded silicosan particles.

**MATERIALS AND METHODS**

**Materials**

Aripiprazole (ARP) was supplied from Alembic Pharmaceuticals Limited, Karakhadi, India. Tetraethyl orthosilicate (98%;
TEOS), Poloxamer 407, dialysis tubing cellulose membrane (molec-
ular weight cut of 14,000 g/mol), dichloromethane, and methanol
(HPLC grade) were purchased from Sigma-Aldrich, USA. Chitosan
hydrochloride (CS-HCl) was gifted from Zhejiang Chemicals
Import and Export Corporation, China. Tween 80 and ethanol were
purchased from El-Nasr Pharmaceutical Chemicals Company,
Egypt. Cetyltrimethylammonium bromide (CTAB) was obtained
from Bio Basic Inc., Canada. Isopropyl alcohol was purchased from
SDS, France. All other reagents were of analytical grade and were
used as received. All water used was deionized, bi-distilled water.

**Preparation of ARP-Loaded Silicosan Particles**

For the preparation of ARP-loaded silicosan particles, the
drug was dissolved in isopropyl alcohol (0.5 mL) and then it was
mixed with 1 mL TEOS using a vortex mixer (Reax Top Vortex
Mixer, Heidolph, Germany). Then, the mixture was added to
the CS-HCl aqueous solution (0.2% w/v). The preparation was
then left overnight at room temperature until the formation of a
gel structure. The compositions for the prepared ARP-loaded
silicosan particles are listed in Table I.

For the preparation of surfactant containing ARP-loaded
silicosan particles, accurately weighed amount of the surfactant
(1% w/v/CTAB, Tween 80, or Poloxamer 407) was first dissolved
in the CS-HCl solution, then the same procedure conducted for
the preparation of ARP-loaded silicosan particles was applied.

Finally, the resulting gel structures were placed in wide
mouth bottles, frozen at −80°C for 24 h, then lyophilized in a
Christ freeze dryer (ALPHA 2-4 LD plus, Germany) under a
temperature of −80°C and vacuum of 7 × 10⁻² mbar for 24 h.
The obtained powders from the lyophilized samples were
stored in desiccators until use.

**Evaluation of the Prepared ARP-Loaded Silicosan Particles**

**Fourier Transform Infrared Spectroscopy (FTIR)**

The FTIR spectra were recorded for ARP, CS-HCl,
TEOS, Tween 80, Poloxamer 407, CTAB, lyophilized
ARP-free, and ARP-loaded silicosan particles using
Bruker FTIR spectrophotometer (Model 22, Bruker, UK)
using the KBr disk technique. The FTIR measurements
were performed in the scanning range of 4000–400 cm⁻¹
at ambient temperature.

**Scanning Electron Microscope (SEM) Imaging Coupled with
Energy Dispersive X-ray Analyzer (EDX)**

Morphology of selected lyophilized ARP-loaded silicosan
particles were observed using a SEM (Jeol JSM-6400; JEOL Ltd.,
Tokyo, Japan). The samples were fixed using double-sided
adhesive tape on a brass stub and then it was subjected to
sputter-coated with a thin layer of gold. Imaging of the samples
was operated using SEM at an excitation voltage of 20 kV.
Quantitative chemical analysis on particle surfaces was performed
by energy dispersive X-ray analyzer (EDX).

**Entrapment Efficiency**

Fixed amount of the prepared ARP-loaded silicosan
particles was mixed with 1 mL ethanol to dissolve the non-
entrapped drug crystals, followed by centrifugation for 15 min
at 20,000 rpm (Sigma 3-30 K, Spincontrol Comfort, Ger-
many). The residue was then separated and dissolved in
dichloromethane and assessed for entrapment ef-
ciciency. The entrapment efficiency was calculated using the
following equation:

\[
\text{Entrapment efficiency} \left(\%\right) = \left(\frac{\text{amount of drug in the silicosan particles}}{\text{amount of added drug}}\right) \times 100
\]

**Evaluation of the Lyophilized ARP-Loaded Silicosan Particles**

**Determination of Freeze-Drying Process Yield**

The obtained silicosan powder after the freeze-drying
process was weighed and the process yield was calculated by
the following equation:

\[
\% \text{yield} = \left(\frac{\text{recovered mass}}{\text{mass entered in the freeze dryer}}\right) \times 100
\]

**Determination of Particle Size and Zeta Potential**

The particle size and zeta potential of the prepared
lyophilized ARP-loaded silicosan particles were measured
using Mastersizer 2000 and ZetaSizer Nano ZS, respectively (Malvern Instruments, UK). The lyophilized powder of ARP-loaded silicosan particles was dispersed in distilled water prior to measurement.

In vitro Drug Release

The in vitro ARP release from selected ARP-loaded silicosan particles was evaluated at both gastric and intestinal pH using USP II dissolution apparatus (Hanson SR8-Plus, USA) following a pH change method. Accurately weighed amount of lyophilized ARP-loaded silicosan particles (equivalent to 10 mg ARP) were dispersed into 300 mL of 0.1 N HCl (pH 1.2). After 2 h, 100 mL of trisodium phosphate (0.26 M) were added to each vessel to achieve a pH of 6.8 and the experiment was run for further 6 h (21). The temperature of the medium was maintained at 37 ± 0.5°C while the speed of the paddle was adjusted at 50 rpm throughout the experiment. At definite time intervals, 5 mL samples were withdrawn and filtered using a dissolution filter (10 μm UHMW polyethylene Cannula dissolution filter, Hanson, USA) and replaced with fresh medium in order to maintain sink conditions. The in vitro release of 2 mL ARP aqueous suspension (prepared by direct dispersion of 10 mg ARP in 2 mL distilled water) was also done for comparison using the same procedure mentioned above. Samples were subjected to HPLC analysis using a sensitive, selective, and accurate HPLC method that was developed and validated before the study for determination of ARP concentrations in collected samples. All chemicals and reagents were of analytical grade, solvents used were of HPLC grade. HPLC system consisting of isocratic pump LC-10 AD and a U/VIS detector SPD-10A was connected to a SCL-10A integrator; Knauer advanced scientifi c UV 2250 (Berlin, Germany). The analytical column was Eurosphere C18 column, 250 mm × 4.6 I.D. mm, particle size 5 μm (Berlin, Germany). The isocratic mobile phase comprised methanol:water (90:10, v/v). The mobile phase was filtered and degassed. The flow rate was set at 1.2 mL/min and the column effluent was monitored continuously using UV detection at 254 nm, the injection volume was 20 μL.

X-ray Diffractometry (XRD)

X-ray diffraction pattern was recorded using Philips PW-1050 diffractometer equipped with filter Ni, Cu Kα radiation. The tube was operated at a voltage of 40 kV and a current of 20 mA. The XRD patterns of the plain ARP, CS-HCl, Poloxamer 407, CTAB, ARP-free, and ARP-loaded silicosan particles were recorded.

Differential Scanning Calorimetry (DSC)

The thermal behavior of the pure ARP, CS-HCl, Poloxamer 407, CTAB, ARP-free and ARP-loaded silicosan particles were traced using Shimadzu differential scanning calorimeter (DSC-30, Shiga, Japan). For each scan, 2 mg of each sample were weighed and placed in a hermetically sealed aluminum pan. The samples were then heated in a temperature range of 25–350°C at a constant heating rate of 10°C/min under a nitrogen atmosphere.

### Table I. Composition and Characterization for Silicosan Particles

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Surfactant</th>
<th>TEOS to Chitosan HCl ratio (V/V)</th>
<th>Entrapment efficiency (%)</th>
<th>Characterization of lyophilized aripiprazole-loaded silicosan particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Process yield (%)</td>
<td>Particle size (μm)</td>
</tr>
<tr>
<td>SC-1</td>
<td>–</td>
<td>1:1</td>
<td>10.13 ± 5.17</td>
<td>–</td>
</tr>
<tr>
<td>SC-2</td>
<td>–</td>
<td>1:2</td>
<td>69.42 ± 5.12</td>
<td>14.859 ± 0.106</td>
</tr>
<tr>
<td>SC-3</td>
<td>–</td>
<td>1:6</td>
<td>37.720 ± 1.05</td>
<td>–</td>
</tr>
<tr>
<td>SC-2-2</td>
<td>–</td>
<td>1:2</td>
<td>24.335 ± 1.82</td>
<td>–</td>
</tr>
<tr>
<td>SC-2-TW</td>
<td>Tween 80</td>
<td>1:2</td>
<td>48.840 ± 1.11</td>
<td>62.97 ± 2.24</td>
</tr>
<tr>
<td>SC-2-Pix</td>
<td>Poloxamer 407</td>
<td>1:2</td>
<td>68.855 ± 2.41</td>
<td>64.97 ± 1.54</td>
</tr>
<tr>
<td>SC-2-CTAB</td>
<td>CTAB</td>
<td>1:2</td>
<td>65.050 ± 6.21</td>
<td>86.59 ± 0.15</td>
</tr>
</tbody>
</table>

**All the formulations were prepared using 5-mg drug except for SC-2-2, where 10-mg drug was used in its preparation. CTAB, cetyltrimethylammonium bromide; RE, release efficiency**

**The release efficiency for drug suspension was 18.96 ± 2.96%**
Flow Properties of Lyophilized ARP-Loaded Silicosan Particles

Two methods were used for powder flowability measurement. The first method (Hauser’s ratio and Carr’s index) was used to calculate the bulk density and tapped density of the powder blends (22). The lower the Hausner ratio and the Carr index, the better is the powder flowability. The second method was the characterization of flowability by measuring the angle of repose according to the fixed funnel and free standing cone method (23). This angle represents the maximum possible angle between the surface of a powder pile and the horizontal plane.

In vivo Studies in Rabbits

Study Design. The in vivo studies were carried out to compare the bioavailability and pharmacokinetic parameters of 10 mg ARP from two different treatments in white New Zealand male Albino rabbits (2.5–3 kg) using a non-blind, cross-over design. Three rabbits were randomly assigned to each treatment group. Food was withdrawn 10 h prior to the study with water ad libitum. In the early morning, the assigned treatments were administered. In treatment A, rabbits received ARP aqueous suspension (prepared by direct dispersion of ARP in distilled water). In treatment B, rabbits received ARP-loaded silicosan particles prepared using CTAB (SC-2-CTAB). Both treatments were dispersed in 2-mL distilled water to achieve an amount of ARP equivalent to 10 mg ARP per kg body weight of the rabbit, administered through an intragastric tube. Blood samples (2 mL) were withdrawn retro-orbitally at 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 10 h after treatment administration. Blood samples were collected into heparinized tubes and plasma was obtained by centrifugation at 4000 rpm for 15 min. The plasma was pipetted into glass tubes and frozen at −20°C until analysis. All animal experiments were approved by the Research Ethics Committee (REC) for Animal Subject Research at the Faculty of Pharmacy, Cairo University, Egypt and operated according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Chromatographic Conditions. Chromatographic separation was performed on a JASCO HPLC system equipped with a PU-20180 Plus pump and a DG-2090-54 degasser. The elution was achieved on an Inertsil RP-HPLC ODS column (4.6 × 250 mm, 5 μm) using isocratic elution. The mobile phase was composed of methanol and water (90:10 v/v) with a detection wavelength of 254 nm. ARP was eluted at 16.5 min while the internal standard (aceclofenac) was eluted at 11.5 min.

Validation. All assays were performed in independent triplicates. The lower limit of quantification was 0.1 ng/mL and a linear response ($r^2 = 0.992$) was obtained.

Plasma Analysis. Sample preparation was performed using a modified protein precipitation method in which 300 μL of the plasma were mixed with 10 μL of the internal standard (to give a concentration of 10 ng/mL) and 700 μL of acetone. The samples were left to stand for 15 min with vortex mixing. Then, sample-precipitated proteins were separated by centrifugation at 6000 rpm. The supernatant (600 μL) was withdrawn and evaporated to dryness. Samples were then reconstituted in the mobile phase (30 μL) prior to injection.

All frozen plasma samples obtained from the rabbits after receiving treatments A and B were thawed at ambient temperature and assayed as described above without the addition of ARP.

Pharmacokinetic Analysis. Data from plasma analysis were analyzed for each rabbit using WinNonlin® (version 1.5, Scientific consulting, Inc., Cary, NC). Non-compartmental analysis was pursued and the pharmacokinetic variables: $C_{max}$ (maximal drug concentration; ng/mL), $T_{max}$ (time for maximal drug concentration; h) and AUC$_{0-\infty}$ (area under the curve; ng.h/mL) were generated.

The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln2/k$. The relative bioavailability ($f_{rel}$) was calculated for ARP-loaded silicosan particles (SC-2-CTAB) relative to ARP aqueous suspension as $\text{AUC}_{\text{SC-2-CTAB}}/\text{AUC}_{\text{susp}}$. All results were expressed as mean $n = 3 ± SD$.

Statistical Analysis

Statistical analysis of the in vivo studies were based on untransformed values for $C_{max}$ and AUC variables and observed values for $t_{1/2}$. The nonparametric Signed-Rank Test (Mann-Whitney’s test) was used to compare $T_{max}$ between the two treatment groups.

RESULTS AND DISCUSSION

Preparation and Characterization of ARP-Loaded Silicosan Particles

In our study, isopropyl alcohol was used to dissolve the drug, as it is miscible with TEOS unlike methanol and ethanol. Preliminary studies were conducted in order to determine the feasibility of preparing ARP-loaded silicosan particles with an acceptable particle size and entrapment efficiency-values, with the aid of CS-HCl. Successful formulations were obtained at room temperature without the need of elevated temperature or any other harsh conditions that may affect sensitive drugs. Three different TEOS-CS-HCl volume ratios (1:1, 1:2, and 1:6 v/v) were prepared containing fixed ARP concentration for the prepared ARP-loaded silicosan particles. Furthermore, ARP-loaded silicosan particles were prepared with the incorporation of Tween 80, Poloxamer 407, or CTAB.
Evaluation of the Prepared ARP-Loaded Silicosan Particles

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were assessed to demonstrate the interaction between the components in the formulation (Fig. 1a). The FTIR spectrum of TEOS showed absorption bands at 2976, 2927, and 2893 cm\(^{-1}\) due to aliphatic C–H stretching. The band located at 1086 cm\(^{-1}\) is related to the stretching vibrations of C–O groups. The CS-HCl showed a broad peak in its FTIR spectrum at 3439 cm\(^{-1}\) contributing to the stretching of NH\(_2\) and OH groups. The stretching vibrations of C–O group for the CS-HCl appeared at 1091 cm\(^{-1}\) and at 1024 cm\(^{-1}\). The bending vibrations of aliphatic C–H stretching were located at 2888, 2849, and 2767 cm\(^{-1}\).

The FT-IR spectrum of Tween 80 showed intense bands at 2922 and 2869 cm\(^{-1}\) due to the aliphatic C–H stretching. The O–H group showed absorption band at 3423 cm\(^{-1}\). The band at 1735 cm\(^{-1}\) can be attributed to the O–C=O group while the band at 1643 cm\(^{-1}\) can be attributed to the stretching of the C=C group. The band at 1107 cm\(^{-1}\) is assigned to asymmetric C–O stretching vibrations. Poloxamer 407 showed a broad band at 3442 cm\(^{-1}\) corresponding to the O–H group. The absorption bands being noticed at 2968 and 2888 cm\(^{-1}\) are due to aliphatic C–H stretching. The peak appearing at 1109 cm\(^{-1}\) is assigned to C–O stretching vibrations. The FTIR spectrum of CTAB revealed strong absorption C–H stretching bands at 2918 and 2849 cm\(^{-1}\). The band at 1473 cm\(^{-1}\) is due to C–N stretching mode.

The interaction between TEOS and CS-HCl can be detected from the evolution of new IR band for Si–N in the final material. This band can be attributed to the new covalent bond formed between the Si–O–CH\(_2\)–CH\(_3\) groups of TEOS and C–NH\(_2\)HCl groups of CS-HCl. The presence of the band for the Si–N group at 953, 955, 955, and 961 cm\(^{-1}\) for formulation prepared without surfactant, with Tween 80, with Poloxamer 407, and with CTAB, respectively, clearly confirms the interaction between TEOS and CS-HCl to form the silicosan particles, as shown in Fig. 1a.

The FTIR spectra for formulations prepared using Poloxamer 407 or CTAB confirm the presence of the bands

![Fig. 1. a FTIR spectra for (1) chitosan HCl (CS-HCl), (2) TEOS, (3) Tween 80, (4) Poloxamer 407, (5) CTAB, drug-free formulations: (6) SC-2, (7) SC-2-TW, (8) SC-2-Plx, and (9) SC-2-CTAB, ARP-loaded formulations: (10) SC-2, (11) SC-2-TW, (12) SC-2-Plx, (13) SC-2-CTAB, and (14) aripiprazole (ARP). Chemical structure for the formulated silicosan particles: b prepared without surfactant or using Poloxamer 407 or CTAB and c prepared using Tween 80]
for the active groups of the surfactants. Such results confirm that Poloxamer 407 or CTAB did not form chemical interaction with TEOS or CS-HCl and gives silicosan structure demonstrated in Fig. 1b. On the other hand, with the data obtained, it was found that Tween 80 was involved in the interaction between TEOS and CS-HCl. The disappearance of the O=C=O band for Tween 80 at 1735 cm$^{-1}$ gave evidence for chemical interaction between this group and the C=NH$_2$HCl group of CS-HCl resulting in formation of the C=N group at 1541 cm$^{-1}$. This result reveals that the composition was changed due to introduction of Tween 80 to give the silicosan structure demonstrated in Fig. 1c.

The FTIR spectrum of ARP (Fig. 1a) shows many sharp absorption bands representing the different functional groups in the drug molecule. The absorbance bands at 3437 and 3348 cm$^{-1}$ are corresponding to O–H and N–H stretching, respectively. The absorption band at 3112 cm$^{-1}$ represents aromatic C–H stretching while the bands at 2978 and 2930 cm$^{-1}$ are due to aliphatic C–H stretching. The N–C=O group showed intense peak at 1674 cm$^{-1}$. The bands due to stretching of C–N and C–Cl groups appeared at 1161 and 792 cm$^{-1}$, respectively. The aromatic C=C stretching appeared at 1626, 1492, and 1454 cm$^{-1}$.

ARP retained its characteristic peaks in the prepared formulations confirming that it was not involved in the interaction between TEOS and CS-HCl (Fig. 1a). The characteristic amide group (N–C=O) for ARP located at 1674 cm$^{-1}$ was shifted to lower frequencies, to 1634, 1636, 1634, and 1633 cm$^{-1}$ for formulation prepared without surfactant, with Tween 80, with Poloxamer 407 and with CTAB, respectively. The shift of the amide group for the ARP can be related to the hydrogen bond formation between this group and the O–H group in CS-HCl and/or terminal silanol groups in the silicosan particles, which confirm binding between CS-HCl and ARP.

These results conveyed a clear picture about interaction between TEOS and CS-HCl and binding the new prepared particles to ARP, which is specifically important for orally administered silica-based drug delivery system. These changes suggested that the formation of hydrogen bonds between ARP and the silicosan particles. This situation may be due to the establishment of different types of host–guest interactions (H-bond) between ARP and silicosan particles (24).

**Scanning Electron Microscope Imaging**

The morphology of ARP-loaded silicosan particles was investigated by SEM (Fig. 2). It could be clearly seen that the different structures of silicosan network are formed upon changing the surfactant type. Fig. 2a shows that preparing ARP-loaded silicosan particles in the absence of any surfactant cause the silicosan network to appear as interconnected mesh with no well-defined particles. On the other hand, upon using Tween 80, the silicosan network appeared as agglomerated porous structures covered with some connected particles forming ramified long chains (Fig. 2b). Figure 2c clearly shows that ARP-loaded silicosan particles prepared using poloxamer consists of tiny particles that seems to be connected in the shape of randomly oriented coagglomerates. Using CTAB as a surfactant to prepare ARP-loaded silicosan particles produced spherical

![Fig. 2. SEM images and EDX figures (intensity in counts versus energy in keV) for a SC-2, b SC-2-TW, c SC-2-Plx, and d SC-2-CTAB](image-url)
particles aggregated in semi-spherical agglomerates interconnected with short chains (Fig. 2d).

The presence of silica atoms, ARP, CS-HCl, and surfactants in different ARP-loaded silicosan particles were further confirmed by the elemental analysis (EDX) (Fig. 2). The EDX figures indicate the elemental analysis of the ARP-loaded silicosan particles. It is obvious in Fig. 2 that the oxygen, carbon, nitrogen, and silica atoms exist in all the investigated silicosan particles as expected. The elemental analysis also yielded the atomic percentage of Si element which were equal to 1.76% for SC-2 (ARP-loaded silicosan particles with no surfactant), while the approximate Si percentages were 20.93, 17.74, and 34.98% for SC-2-TW, SC-2-PLX, and SC-2-CTAB, respectively, confirming successful preparation of the fabricated silicosan particles. EDX data showed increase in the intensity of silica atom peak upon including surfactants into ARP-loaded silicosan particles (SC-2-TW, SC-2-PLX, and SC-2-CTAB). This could conclude that the interaction between the TEOS and CS-HCl is largely increased when surfactants were included in the fabrication media. Furthermore, SC-2-CTAB formulation prepared using CTAB as surfactant had the highest content of silica (34.98%). This may be attributed to the positive charge of CTAB which may prefer to attract and emulsify the negatively charged TEOS (source of silica atoms) resulting in the formation of silicosan particles with high percentage of silica atoms.

**Entrapment Efficiency of ARP-Loaded Silicosan Particles**

Results of the entrapment efficiency for the prepared ARP-loaded silicosan particles are presented in Table I. Results showed that increasing the TEOS:CS-HCl volume ratio from 1:1 (SC-1) to 1:2 (SC-2) or 1:6 (SC-3) resulted in a significant increase in entrapment efficiency values from 10.1% for SC-1 to 58.3 and 37.7% for SC-2 and SC-3, respectively (p < 0.05). This may be attributed to the increase in the amount of CS-HCl which interacts with TEOS to produce more silicosan particles that will entrap the drug. Increasing the amount of CS-HCl would result in less number of TEOS molecules that will react with single chitosan molecule.

Further increase in TEOS:CS-HCl volume ratio from 1:2 (SC-2) to 1:6 (SC-3) was not coupled with an increase in the entrapment efficiency values of the ARP-loaded silicosan particles, on the contrary, the entrapment efficiency decreased. Such significant decrease in entrapment efficiency (p < 0.05) was attributed to the large volume of the aqueous phase containing CS-HCl added to the TEOS which resulted in dilution of the drug and its precipitation (this was visually inspected during the preparation).

In an attempt to improve the entrapment efficiency value of the prepared formulation, ARP-loaded silicosan particles (SC-2) was modified by increasing the drug loading during preparation from 5 to 10 mg (formulation SC-2-2). Unfortunately, increasing drug-loading concentration did not improve the entrapment efficiency of the drug, but on the contrary, a great declination of drug entrapment value was produced. This may be due to the saturation of the isopropyl alcohol with the drug that was rapidly precipitated upon contact with the aqueous silicosan particles with high percentage of silica atoms.

Among the tested formulations, ARP-loaded silicosan particles (SC-2) with TEOS:CS-HCl volume ratio of 1:2 showed the highest entrapment efficiency value. Therefore, this formulation was selected for further incorporation of surfactants, namely, Tween 80, Poloxamer 407, or CTAB, seeking improvement of the tested parameters.

The entrapment efficiency-values of the obtained silicosan particles prepared in presence of surfactants were enhanced upon using Poloxamer 407 or CTAB during the formulation (p < 0.05) due to their solubilization effect on the drug (25). The highest value for entrapment efficiency (68.855 ± 2.403%) was recorded for SC-2-Plx. Previous studies had proven the superiority of Poloxamer 407 in increasing the drug entrapment and loading capacities as it provides great stabilization during particle formation (26). On the other hand, using Tween 80 caused a significant decrement in the entrapment efficiency of the obtained formulation (p < 0.05) due to its consumption in the formation of the new chemical structure of silicosan particles (Fig. 2c).

A noticeable remark to be documented is that upon the use of Tween 80 or Poloxamer 407 as a surfactant in preparing the silicosan particles, faster gel formation (less time for gel formation; less than 24 h) was observed.

Silicosan particles containing Tween 80 showed faster gel formation than that containing Poloxamer 407. On the other hand, ARP-loaded silicosan particles containing CTAB did not affect the gelling time compared to formulation lacking surfactant. A possible explanation is the high HLB-values of Tween 80 and Poloxamer 407 relative to CTAB, favoring greater mixing of the phases (organic TEOS phase and aqueous CS-HCl phase). HLB-values for Tween 80, Poloxamer 407, and CTAB are 15, 18-23, and 10, respectively. Although Poloxamer 407 has higher HLB value than that of Tween 80 and was expected to show faster silicosan particles formation than that for formulation containing Tween 80, it showed longer gelling time. This may be attributed to the higher viscosity of the Poloxamer 407 solution that might have caused retardation of phases mixing. Further characterization studies (DSC and XRD) were performed in order to thoroughly investigate the nature and composition of the obtained ARP-loaded silicosan particles.

The promising preliminary study results for SC-2 formulation and its modified surfactant-formulations habilitated them for further subjection to further studies.

**Determination of Process Yield, Particle Size, and Zeta Potential**

Considering the importance of yield value as a significant parameter for scaling-up process, yield values for the prepared formulations were investigated in the current study (Table I). It can be clearly demonstrated in the table the promising high values for this parameter which indicates the success of the applied ARP-loaded silicosan particles preparation method. Yield values ranged from 62.971 ± 2.245 to 86.586 ± 0.145% with the maximum value attended for CTAB containing formulation. The high process yield value for SC-2-CTAB may be due to the high melting point of CTAB (249–253°C) (27) compared to those for Tween 80 (~20.56°C) (28) and Poloxamer 407 (53–57°C) (29) which resulted in a less sticky product.
It was found that silicosan formulation with higher silica atom content (SC-2-CTAB) had the higher particle size value and silicosan particles with lower silica atom content (SC-2) had the lowest particle size value. This could be attributed to the molecular volume for the formed silicosan where increasing the number of reacted TEOS with the chitosan molecule would result in more bulky molecule that would be backed in large volume particles.

The zeta potential value for the lyophilized SC-2 formulation exhibited a negative value due the predominance of silanol group having a larger electron cloud, due to the unshared lone pairs, giving negative charge for the formulation. Such negative zeta potential value for the lyophilized SC-2 was decreased upon using Tween 80 in the formulation due to the interaction of Tween 80 with TEOS. On the other hand, using Poloxamer 407 during the preparation of SC-2-Plx resulted in the formation of ARP-loaded silicosan particles with positive zeta potential value which indicate the presence of CS-HCl that was not involved in the reaction. The SC-2-CTAB preparation possessed a high positive zeta potential value of 33.1 mV due to the presence of the positively charged surfactant, CTAB.

**Determination of Physical State of the Drug Within the Prepared ARP-Loaded Silicosan**

Solubility/dissolution-limited bioavailability can be overcome through the preparation of amorphous drug forms (30). However, the amorphous state can change back to the crystalline state during processing or storage. Stabilization of amorphous drugs through the adsorption in/on materials is gaining much interest (31).

In order to get the benefit from high solubility and dissolution, the challenge of poor stability of amorphous compounds has to be addressed seriously. The interaction of ARP with silicosan particles, either via adsorption and/or integration within the matrix, could change its physical form; especially in the presence of the unique structure of the prepared ARP-loaded silicosan particles represented by their characteristic high surface area. The well-known important role of drug’s crystallinity on its dissolution rate and consequently its increased bioavailability has driven us to explore it. Thus, DSC and XRD were used to determine the crystallinity/amorphous state of the entrapped ARP within the prepared ARP-loaded silicosan particles.

**Differential Scanning Calorimetry (DSC)**

Differential scanning calorimetric analysis was employed to evaluate the molecular state of ARP in the prepared formula. DSC thermograms of ARP, CS-HCl, Poloxamer 407, CTAB, ARP-free and ARP-loaded silicosan particles are shown in Fig. 3a. As illustrated in Fig. 3a, the free ARP was characterized by a single, sharp melting endothermic peak at 183.9°C, corresponding to its melting point, revealing a crystalline anhydrous substance typical behavior. CS-HCl thermogram showed two endothermic peaks; one at 91.73°C related to water loss associated with hydrogen-bonding to the hydrophilic groups of CS-HCl, and the other at 221.2°C, in addition to an exothermic peak at 50°C.
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218.1°C, indicating its degradation (32). Poloxamer 407 shows an endothermic peak at 58.01°C corresponding to its melting point. The DSC thermogram of CTAB demonstrates two endothermic peaks at 61.58 and 433.3°C. Interestingly, all the peaks of the tested excipients disappeared in the prepared ARP-free silicosan particles, suggesting complete transformation of the excipients to the amorphous state.

All the evaluated ARP-loaded silicosan particles revealed the disappearance of the melting endothermic peak of ARP crystals, thus provided indirect evidence for the effective adsorption of ARP in the internal network of silicosan particles. The DSC thermograms for the medicated formulations were similar to their counterpart non-medicated formulations. The absence of the characteristic peaks of ARP within the medicated formulations confirms its transformation to its amorphous form and its complete entrapment within the prepared silicosan formulations (33). These results supported that the ARP crystals changed to the amorphous (34) or molecular state (35) during the loading procedure.

X-ray Diffractometry (XRD)

Figure 3b shows XRD diffractograms of ARP, CS-HCl, Poloxamer 407, CTAB, ARP-free, and ARP-loaded silicosan particles. XRD data confirm the obtained results from DSC analysis. The XRD diffractograms for the medicated and non-medicated formulations showed amorphous structure. ARP lost its crystallinity and fully integrated within the prepared ARP-loaded silicosan particles.

The diffraction pattern of ARP powder revealed principle intense peak at 11.07° and intense peaks at 6.9°, 6.3°, and 3.6°, suggesting that the drug existed as crystalline material. The powder XRD of ARP-loaded silicosan particles showed the typical halo pattern due to amorphous nature of the silicosan particles in the range 4–80° and the peaks attributable to the crystalline ARP was not available. This typical diffuse pattern indicated the entirely amorphous nature of ARP in the system. According to Williams et al., lack of crystallinity is an added evidence for the formation of inclusion complex (36). This proves that silicosan particles kept their structure after loading with ARP and the included ARP was not arranged in a crystalline form.

Flow Properties of the Prepared ARP-Loaded Silicosan Formulations

Powder flow properties are important aspects in processing operations, such as flow from hoppers, mixing, and compression. A uniform flow from the hoppers into the die cavity ensures uniform tablet weight and content uniformity. Poor powder flow presents a major problem to the pharmaceutical industry (37). The effect of the three surfactants on the flow and dissolution properties of the prepared ARP-loaded silicosan particles were studied. The flowability of the powder blends was determined using Hausner’s ratio, Carr’s index, and angle of repose. Hausner’s ratio (HR) was related to the inter particle friction; powders with a low inter particle friction had a ratio of approximately 1.25 indicating a good flow. Generally, powders with Carr’s index below 25% have very good flow properties (37). Powders with angle of repose above 50° have unsatisfactory difficult flow properties, while those with values of 25–40° represent reasonable flow potential, whereas minimum angles close to 25° correspond to very good flow properties (38). From the results presented in Table I, all the prepared ARP-loaded silicosan particles showed acceptable values for Hausner’s ratio, Carr’s index, and angle of repose (Table I). Such free-flowing powder properties prevents particles from aggregation, ensures great surface area allowing the particles to be rapidly diffused and easily dispersed upon contact with the desired vehicle (39). Such results would refer to the efficacy of the resulted lyophilized powder to be filled into hard gelatin capsule shell.

In vitro Drug Release

The ARP release profiles from different silicosan hosts were compared with that of pure ARP and shown in Fig. 4. Great enhancement of ARP release from ARP-loaded silicosan particles compared with ARP alone was observed. The great enhancement of ARP release from ARP-loaded silicosan particles could be attributed to both the high specific surface area that cause both high drug adsorption/loading and for high drug release rate (40) and the presence of free unreacted silanol present on the surface groups on the silicosan surface which renders the particles’ surface hydrophilic providing good wettability of the particles. Silanol groups can interact with proton donor/acceptor groups of the molecules hosted in the silicosan through hydrogen bonding (as confirmed in the FTIR results) which are easily broken upon hydrating in the release medium. Water enters into the silicosan structure and dislocation of the guest molecules occurs towards the external environment by diffusion (41). This dissolution behavior can also be attributed to the following: first, the increase in surface area of ARP by its adsorption on the surface of silicosan particles that provided larger effective dissolution area. Second, the existence of ARP in an amorphous state (confirmed by DSC and powder XRD studies), as the witnessed dissolution improvement of ARP was associated with alteration in the solid-state properties of the drug (42). In general, the amorphous structure of any hydrophobic compound has higher dissolution rate than the crystalline form, in aqueous media (43). Third, the wettability of the hydrophilic silicosans, where silica particles have a hydrophilic surface owing to the presence of the silanol groups; therefore, it has good wettability for aqueous media.

All in vitro release data of ARP from different ARP-loaded silicosan particles were evaluated, and the RE% was calculated from the area under the release curve at time t. It is expressed as the percentage of the area under the release curve to the rectangle corresponding to 100% drug release, for the same total time, according to the following equation (44):

\[ \text{RE}\% = \frac{\int y \times dt}{y_{100} \times t} \times 100 \]

where, RE is the release efficiency, y is considered the percentage drug released at certain time t and y_{100} is the 100% drug release at time t. The release profiles of ARP from surfactant enriched ARP-loaded silicosan particles show that CTAB containing formulations had an improved drug release profile compared to the corresponding formulation lacking surfactant. This
could be attributed to the high surface charge of the CTAB containing particles (+33.1 ± 3.11 mV) as well as the solubilization effect for CTAB, which resulted in greater dispersion in the particles within the medium with greater surface area exposed to the release medium and greater, hence, enhanced drug solubilization and release. On the other hand, Tween 80 containing formulations had a similar drug release profile with that of the corresponding formulation lacking surfactant. This could be attributed to the involvement of Tween 80 in the interaction between TEOS and CS-HCl as previously proven from FTIR analysis (Fig. 1). Moreover, Poloxamer 407 containing formulations had a slower drug release profile compared to that of the corresponding formulation lacking surfactant. This can be attributed to the hydrophilic nature of Poloxamer 407. Upon contact with release medium at 37°C, excessive hydrogen bonding between water molecule and ethereal oxygen of Poloxamer 407 occurs forming a highly viscous gel layer resulting in drug release retardation (45).

Release efficiency values of the surfactant enriched ARP-loaded silicosan particles were calculated to be 62.65 ± 0.82, 81.79 ± 2.49, and 95.72 ± 0.74%, for Poloxamer 407, Tween 80, and CTAB containing formulations, compared to 82.67 ± 3.01 for the surfactant-free formulation (SC-2). Although slower release was expected from Tween 80 containing formulations owing to the greater particle size and subsequent smaller area of the particles compared to SC-2, no significant difference was observed between the release efficiency values of SC-2 and SC-2-TW (p > 0.05). This suggests that the increase in particle size was compensated by hydrophilic nature of Tween 80 that caused drug solubilization. As expected, the RE for ARP plain powder was very low (18.96 ± 2.96%), due to its hydrophobic nature and poor wettability. SC-2-CTAB silicosan particles with the largest value for release efficiency was chosen for in vivo study.

**In vivo Study**

To assess the impact of loading ARP into the silicosan particles (SC-2-CTAB) on the pharmacokinetic parameters and the in vivo bioavailability of ARP, rabbits were administered a dose of 10 mg/kg of SC-2-CTAB through the oral route and results were compared to an equal dose of ARP oral suspension (Fig. 5). The obtained pharmacokinetic results showed that the relative bioavailability for ARP-loaded silicosan particles (SC-2-CTAB) was 66% higher relative to the oral suspension (AUC$_{0-10h}$ was 16.38 ± 3.21 and 27.23 ± 2.35 ng.h/mL for ARP powder and SC-2-CTAB formulation, respectively). The maximum ARP plasma concentration reached following the administration of the silicosan particles was 1.7-fold higher compared to that of the ARP suspension (7.69 ± 1.93 and 4.53 ± 1.40 ng/mL, respectively), reached within the same time (t$_{max}$ = 1.5 h for both treatments). The significantly higher values of the extent parameters (C$_{max}$ and AUC$_{0-10h}$) were observed following the administration of ARP-loaded silicosan particles (SC-2-CTAB) is attributed to the enhanced solubility and dissolution of ARP in the silicosan particles (p < 0.05). No significant difference was observed in the values of t$_{1/2}$ following both treatments (8.94 ± 7.70 and 8.83 ± 6.27 h for drug powder and
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SC-2-CTAB formulation, respectively; \( p > 0.05 \). This is consistent with the pharmacokinetic theory, where the increase in absorption should not affect drug elimination (46).

CONCLUSION

In the present work, silicosan particles, novel delivery carriers, for enhancement of the oral performance of aripiprazole (ARP) were designed. The FT-IR study confirmed the interaction between tetraethyl orthosilicate (TEOS) and chitosan-HCl in order to form the silicosan particles. Furthermore, among the used surfactants, only Tween 80 was found to interact with silicosan formulations. The inclusion of ARP in silicosan particles improved tremendously its dissolution in simulated gastric fluid. Moreover, investigation of ARP state in these novel carriers revealed that the loaded drug appears in a molecular amorphous state. ARP-loaded silicosan particles prepared using CTAB with highest silica atom content had the highest enhancement effect on the drug dissolution. The prepared ARP-loaded silicosan particles succeeded to enhance the ARP bioavailability after oral administration to rabbits in comparison with that of plain drug suspension. The association of the unique-structure of silicosan and the molecular state of ARP presents a potential candidate for delivering the poorly water soluble, ARP, with enhanced oral bioavailability.

COMPLIANCE WITH ETHICAL STANDARDS

All animal experiments were approved by the Research Ethics Committee (REC) for Animal Subject Research at the Faculty of Pharmacy, Cairo University, Egypt and operated according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Conflict of Interest The authors declare that there is no conflict of interest.

REFERENCES