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Formulation of risperidone in floating microparticles to alleviate its extrapyramidal side effects

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ABSTRACT

Risperidone is effective in the treatment of positive as well as negative symptoms of schizophrenia. But, there is a strong correlation between plasma levels of risperidone and its adverse effects.
Objective: This study aimed to develop risperidone in floating microparticles to overcome its extrapyramidal side effects.
Methods: Floating microparticles were prepared using Eudragit S100, hydroxypropylmethyl cellulose (HPMC), Gelucires (Gelucire 43/01 pellets, Gelucire 44/14 and Gelucire 50/13), Geleol mono and diglyceride NF, glyceryl monostearate, Compritol 888 ATO, methyl-betacyclodextrin (MβCD) and hydroxypropyl-betacyclodextrin (HPβCD), by emulsion solvent diffusion technique. *In-vitro* experiments were conducted to optimize formulation parameters regarding floating ability, yield value, drug loading and *in-vitro* release properties. The best formula was investigated for its *in-vivo* floating ability and for its pharmacokinetics as well as its extrapyramidal side effects in human volunteers.
Results: The optimized floating microparticles showed promising *in-vitro* experiment performance with floating ability up to 95.93% for 12 h. Also, this floating ability was confirmed using *in-vivo* x-ray studies. Pharmacokinetics studies revealed significant ($p < 0.05$) lower C_{max}, longer T_{max} and higher AUC values for the optimized formula compared to the marketed oral product (Risperidal® 4 mg tablets) indicating gradually release properties which lead to high treatment efficacy of the drug with obvious reduced extrapyramidal side effects.
Conclusion: These results proved that formulating risperidone as floating microparticles is a suitable dosage form for overcoming risperidone side effects.

1. Introduction

Schizophrenia is a severe and disabling mental illness. It affects approximately 1% of the population worldwide and it is a chronic, severe disorder, lacking curative treatment. The suicide rate in schizophrenia is as high as 9–13%, with the incidence of suicide attempt reaching 50% of diagnosed patients over a lifetime. The onset of schizophrenia usually occurs around 18–25 years of age and is often preceded by premorbid behavioral deviations, such as social withdrawal and affective changes [1].

The symptoms and severity can vary over individual with schizophrenia. The symptoms are commonly divided into three types, namely; positive symptoms, negative symptoms and cognitive deficits [2–4].

Antipsychotics can be generally categorized into first generation antipsychotics (FGAs, formerly known as 'typical' antipsychotics) and second generation antipsychotics (SGAs, formerly known as 'atypical' antipsychotics).

Noncompliance to antipsychotic drugs has been a major problem since long [5]. Noncompliant psychiatric patients suffered an almost double re-hospitalization from relapse, reducing quality of life and increased economic burden [6]. Noncompliance to antipsychotic drugs was mainly caused by their side effects [7], including dose-dependent cardiac arrest deaths [8] and extrapyramidal side effects. For schizophrenia, good tolerance is known to be associated with good compliance and a higher therapy success rate.

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List of abbreviations

AUC	Area under curve
AUMC	Area under the first moment curve
CDs	Cyclodextrins
C_{max}	Maximum plasma concentration
DSC	Differential scanning calorimetry
GIT	Gastrointestinal tract
GMS	Glyceryl monostearate
GRDF	Gastro retentive dosage form
HLB	Hydrophilic lipophilic balance
HPLC	High performance liquid chromatography
HPMC	Hydroxypropylmethyl cellulose
HPβCD	Hydroxypropyl-beta-cyclodextrin
MRT	The mean residence time
MβCD	Methyl-beta-cyclodextrin
PVA	Polyvinyl alcohol
Q_f	Mass of Floating hollow microparticles
Q_s	Mass of Settled hollow microparticles
RE%	Release efficiency
rpm	Rotation per minute
SEM	Scanning electron microscopy
t_{50}	Half life time
XRD	X-ray diffraction

Risperidone is a potent antipsychotic drug, structurally related to atypical antipsychotics. It is used in treatment of schizophrenia and acute bipolar mania. Risperidone has a low propensity of extrapyramidal side effects [9–13]. It is effective in treatment of both negative and positive symptoms of schizophrenia. For risperidone, a dose-related increase in extrapyramidal side effects was indeed observed [14,15]. There was a strong association between plasma levels of risperidone and its adverse effects [16,17].

The treatment of schizophrenia using oral antipsychotic drugs dates back to the mid-1950s. Administration of antipsychotics *via* the oral route offered various advantages such as easiness of administration and portability of medication [18–20]. The true challenge in the development of an oral controlled release drug delivery system is not just to sustain the drug release but also to prolong the presence of the dosage form within the gastrointestinal tract (GIT) until all the drug is completely released at the desired period of time. Furthermore, sustained release antipsychotic dosage form will be expected to give less C_{max} and thus less extrapyramidal side effects.

Gastro retentive dosage forms (GRDFs) are particularly useful for drugs that are primarily absorbed in the duodenum and upper jejunum segments. Floating microparticles as a type of GRDFs were chosen to formulate risperidone to retain the drug in the stomach as the absorption of this drug mainly takes place in the upper part of the gastrointestinal tract, i.e. duodenum and jejunum [21,22] and as this drug is a weak base with a pKa value of 7.9 [23] which make it suitable candidate to be formulated in such dosage form.

Polymers are generally employed in the development of floating drug delivery systems so as to target the delivery of drug at a specific region in the GIT i.e. stomach [24]. Numerous materials have been studied extensively in the design of drug delivery systems [25].

A number of different substances have been investigated for the preparation of floating microspheres; these materials include polymers of natural origin or synthetic origin and also semi-synthetic substances. Floating microspheres can be prepared by using both hydrophilic and hydrophobic polymers.

One of the polymers preferred in the preparation of microspheres is the polymethacrylates (Eudragits) that synthetic cationic and anionic polymer of dimethylaminoethyl methacrylates, methacrylic acid and methacrylic acid esters in varying ratios (methacrylate copolymers) have been recently received increased attention for preparing modified dosage forms because of their inertness and solubility in relatively non toxic solvents of resins with different properties [26,27].

Much attention has been focused on the use of fats and fatty acids as carriers in floating microspheres drug delivery systems [28,29]. Lipids and waxes are considered as good alternatives to polymers in the design of controlled drug delivery systems due to their advantages like low melt viscosity, thereby obviating the need of organic solvents for solubilization, the absence of toxic impurities such as residual monomers catalyst and initiators, their potential biocompatibility and biodegradability and prevention of gastric irritation by forming a coat around the gastric irritating drug [30].

Among waxy materials, Gelucires are a family of relatively inexpensive materials, Gelucire is a family of vehicles derived from mixtures of mono-, di- and tri-glycerides with polyethylene glycol (PEG) esters of fatty acids. The presence of both hydrophobic glycerides and more hydrophilic PEG esters results in a wide range of hydrophobicity and drug release rates. This versatility makes their use very promising as base materials for the production of sustained-release formulations.

Gelucires are available with a range of properties depending on their hydrophilic lipophilic balance (HLB; 1–18) and melting point (33–65 °C) range. They are inert, semi-solid, waxy amphiphilic excipients that are enormously used in controlled-release matrices in order to enhance the physicochemical properties of the drug. Gelucire can be used for different purposes according to their chemical composition [31]. Gelucires were found to be gastro retentive carrier systems, suitable for both polar and non-polar drugs [30].

Consequently, the rationale of this study was to develop risperidone in a dosage form with the ability to make a significant improvement in patient's psychotic symptoms as well as overcoming the extrapyramidal side effect of this drug for better patient health and quality of life. Therefore, floating controlled release microparticles of risperidone was developed. This formulation will have the advantage of floating dosage forms which decreases the drug peak plasma concentration and maintain drug concentration in plasma within the required range.

2. Materials and methods

2.1. Materials

Risperidone was kindly gifted from Janssen-Cilag, Egypt. Tween 20 was obtained from Rhône-Poulenc, France. Eudragit S100, Eudragit L100 and Eudragit 100-55 were kindly supplied by Evonik Roehm, Darmstadt, Germany. Gelucire 43/01 pellets, Gelucire 44/14, Gelucire 50/13, Geleol mono & diglyceride NF and Compritol 888 ATO were kindly supplied by Gattefosse, France. Glyceryl monostearate (GMS) was obtained from Loba Chemie Pvt. Ltd., Mumbai, India. Hydroxypropylmethyl cellulose with different viscosity (HPMC E4, HPMC E6 and HPMC E15) were kindly gifted from El Kahera Pharmaceuticals, Egypt. Methyl-beta-cyclodextrin (MβCD) and hydroxypropyl-beta-cyclodextrin (HPβCD) were gifted from Roqutte, France. Propyl paraben sodium (internal

standard in HPLC assay) was kindly gifted from Alexandria Company for Pharmaceuticals & Chemical Industries, Egypt. Polyvinyl alcohol (PVA); (viscosity of 4% aqueous solution at 40 °C is 25–32 cps), dichloromethane, ethyl alcohol, barium sulphate and hydrochloric acid were obtained from El-Nasr Pharmaceutical Chemicals, Egypt. HPMC E50, methanol, acetonitrile (HPLC grade) and acetic acid were obtained from Sigma–Aldrich, Germany. Risperidal® tablets (strength of 4 mg, Batch number EFL6S00) were obtained from Janssen-Cilag distributors, Egypt.

2.2. Methods

2.2.1. Preparation of floating microparticles

The floating microparticles were prepared by emulsion solvent diffusion technique. Weighed amount of risperidone, polymers and lipid carrier (Tables 1 and 2) were dissolved or dispersed in a mixture of dichloromethane (8 mL) and ethanol (8 mL) at room temperature. Then, this preparation was introduced to an aqueous solution of polyvinyl alcohol (0.75 w/v%, 200 mL) at 40 °C while stirring using mechanical stirrer at 300 rpm, forming an oil-in-water (o/w) type emulsion. The resultant emulsion was stirred for 1 h at 300 rpm.

Table 1
Composition of risperidone floating microparticles.

Formula code	Formula composition (g)		
	0.1 g risperidone		
	Lipid carrier (0.5 g)	Polymers	
		Eudragit S100	HPMC 50 cps
F1	Glyceryl monostearate	0.9	0.1
F2		0.8	0.2
F3		0.7	0.3
F4	Geleol mono and diglyceride	0.9	0.1
F5		0.8	0.2
F6		0.7	0.3
F7	Gelucire 43/01 pellets	0.9	0.1
F8		0.8	0.2
F9		0.7	0.3
F10	Gelucire 44/14	0.9	0.1
F11		0.8	0.2
F12		0.7	0.3
F13	Gelucire 50/13 pellets	0.9	0.1
F14		0.8	0.2
F15		0.7	0.3
F16	Compritol 888 ATO	0.9	0.1
F17		0.8	0.2
F18		0.7	0.3

Table 2
Composition of modified floating microparticles of risperidone.

Formula code	Formula composition (g)[a]	
	Risperidone (g)	Modifications/additives
F19	0.125	–
F20	0.150	–
F21	0.200	–
F22	0.300	–
F23	0.100	0.2 g HPMC E15
F24	0.100	0.2 g HPMC E6
F25	0.100	0.2 g HPMC E4
F26	0.100	0.360 g HPβCD & 0.2 g HPMC E50
F27	0.100	0.721 g HPβCD & 0.2 g HPMC E50
F28	0.100	0.290 g MβCD & 0.2 g HPMC E50
F29	0.100	0.580 g MβCD & 0.2 g HPMC E50
F30	0.100	0.870 g MβCD & 0.2 g HPMC E50
F31	0.100	1.16 g MβCD & 0.2 g HPMC E50

[a] All formulations contain 0.8 g Eudragit S100 and 0.5 g Compritol 888 ATO.

Subsequently, the formed microparticles were filtered, washed and dried overnight at 40 °C to produce microballoons [32,33].

2.2.2. Characterization of risperidone floating microparticles

2.2.2.1. Buoyancy test for microparticles [34]. In triplicates, microballoons (100 mg) were dispersed in solution composed of HCl (300 mL, pH 1.2 at 37 °C) containing Tween 20 (0.02 w/v%) to simulate gastric conditions [35]. The use of 0.02% Tween 20 was to account for the wetting effect of the natural surface active agents, such as phospholipids in the gastrointestinal tract (GIT) [36]. The mixture was stirred on magnetic stirrer at 100 rpm and 37 ± 0.5 °C. After 12 h, the floating particles were separated by filtration. The sinking particles were separated by filtration. Both particle types were weighed after drying at 40 °C overnight. The buoyancy was determined by the weight ratio of the floating particles to the sum of floating and sinking particles.

$$\text{Buoyancy}\ (\%) = \frac{Q_f}{Q_f + Q_s} \times 100$$

where, Q_f and Q_s are the masses of the floating and settled hollow microparticles, respectively.

2.2.2.2. Percent yield of microparticles. The prepared microparticles were weighed and divided by the total amount of all non-volatile components which were used for the preparation of the microballoons.

$$\%\ \text{Yield} = (\text{Actual weight of product}/\text{Total weight of excipient and drug}) \times 100$$

2.2.2.3. Image analyzer. The surface morphology of microparticles was examined by direct optical measurements using Optika Microscope (Optikam B9 digital camera, Italy).

2.2.2.4. Scanning electron microscopy (SEM). The prepared microparticles were fixed on a brass stub using double-sided adhesive tape and then made electrically conductive by coating, in vacuum, with a thin layer of gold for 30 min and then examined by scanning electron microscope (SEM) operated at 10 kV (JEOL-JXA840A electron probe microanalyzer, Japan) [37].

2.2.2.5. Drug loading in floating microparticles. The drug content of the microparticles was determined by dispersing 5 mg formulation (accurately weighed) in 10 mL ethanol, followed by continuous stirring using a magnetic stirrer to dissolve the drug. After filtration through a Xinxing® filter paper no.101, the drug concentration in the ethanol was determined spectrophotometrically at 280 nm (Shimadzu UV Spectrophotometer (240j/PC), Japan). Each determination was made in triplicate. Calculations were done on basis of the calibration curve of the drug in ethanol. The percentage drug loading was calculated as follows [38]:

$$\%\ \text{Drug loading} = \frac{\text{Actual drug content}}{\text{Weight of microparticles}} \times 100$$

2.2.2.6. Differential scanning calorimetry (DSC). Differential scanning calorimetry (DSC) studies allow the fast evaluation of possible incompatibilities, because it shows changes in the appearance, shift of melting endotherms and exotherms, and/or variations in the corresponding enthalpies of reaction [39,40].

Differential scanning calorimetry (DSC) experiments were carried out to characterize the physical state of risperidone in

microparticles as well as to find the presence of any interaction among the drug and the excipients.

The thermal behavior of risperidone, polymers, physical mixtures and the selected formulae were traced by differential scanning calorimetry (Shimadzu Differential Scanning Calorimetry, DSC TA-50 ESI, Koyoto, Japan). The thermograms were obtained by heating one mg sample in an atmosphere of nitrogen at temperature range of 20–450 °C at constant heating rate of 10 °C/min. The thermograms of the selected formulae were compared to those of plain drug, polymers and physical mixtures of the drug and polymers.

2.2.2.7. X-ray diffraction studies (XRD). X-ray diffraction patterns of risperidone, polymers, physical mixtures and the selected formulae were obtained. The samples were irradiated with Ni filtered Cu K_α radiation with an operating voltage of 45 kV and current of 40 mA. The scanning rate employed was 2°/min over diffraction angle (2θ) range of 3–70°.

2.2.2.8. In-vitro drug release studies from floating microparticles. The release studies were performed using USP type II (paddles) dissolution test apparatus (SR8 PLUS, Handson dissolution tester, USA). Microparticles were placed in the release vessel containing 300 mL 0.1 N HCl of pH 1.2 and Tween 20 (0.02 w/v%) to simulate gastric fluid and maintained at 37 ± 0.5 °C and stirred at 100 rpm [41]. Samples were collected (5 mL) periodically and replaced with a fresh release medium. The concentration of the drug was determined spectrophotometrically at 276 nm (Shimadzu UV Spectrophotometer (240j/PC), Japan). Calculations were done on basis of the calibration curve of the drug in 0.1 N HCl solution of pH 1.2. Drug release studies were done in triplicates. The cumulative percentage of drug released was plotted against time.

Release efficiency (RE) was calculated to compare between the investigated formulations, RE is defined as the area under release curve (AUC) at time t calculated using the trapezoidal rule. It is expressed as a percentage of the area of rectangle corresponding to 100% release, for the same total time according to the following equation [42].

$$R.E. = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100$$

where, y is the amount of drug released at time t and y_{100} donates 100% release.

2.2.2.9. In-vivo floating evaluation of selected risperidone floating microparticles

2.2.2.9.1. Preparation of radio-opaque microparticles for in-vivo studies. The optimized formula which showed the best *in-vitro* performance was chosen for the preparation of radio-opaque microparticles to investigate their *in-vivo* floating ability [43]. Barium sulphate ($BaSO_4$) was selected as a radio-contrast agent to be enclosed in the microparticles. Being highly dense, hence adding $BaSO_4$ to the microparticles lowered their floating ability. Therefore, several trials were done to determine the optimum amount of $BaSO_4$ in microparticles, which will have no effect on the microparticles floating ability and at the same time is enough to allow detection by x-ray. The optimum amount was found to be 1.5% w/w $BaSO_4$ in the tested microparticles. The quantity of barium sulphate added was sufficient to ensure visibility by x-ray, but low enough to enable the microparticles to float.

Barium sulphate loaded microparticles were prepared by adopting the procedure described before except for using barium sulphate instead of the drug.

2.2.2.9.2. In-vivo study of the floating ability of the microparticles. The study was carried out on a healthy male volunteer, free of detectable gastrointestinal diseases or disorders. The volunteer was fasted overnight with free access to water before the experiment, the volunteer ingested the barium sulphate loaded microparticles (40 mg), together with 100 mL of water.

Radiographic examinations were performed to determine the anatomical location of the gastric retention dosage forms [44] by taking a series of x-ray photographs at suitable intervals (Ray mix digital, Italy). The radiographic examinations were performed for the volunteer at zero hr (just before dosing to ensure an empty stomach) and at 1, 4, 8 and 12 h post dosing.

2.2.2.10. Pharmacokinetic studies

2.2.2.10.1. Study design. The protocol of the experiment was approved by the institutional review board of the Research Ethics Committee of Faculty of Pharmacy, Cairo University, Egypt on November 1st, 2011.

The study was performed on two formulae, Risperidal® 4 mg tablets (innovative market product) and the prepared floating microparticles (equivalent to 4 mg risperidone). Six healthy human volunteers, aged between 25 and 40 years with body weight of 55–80 kg after giving informed consent, were randomly divided into two groups, each containing three volunteers. A crossover design was applied on two phases, so that each group received single oral dose of the tested formulae in each phase. A washout period of 2 weeks was left between the two phases.

The volunteers were fasted overnight for 12 h before drug administration and continued fasting until 4 h post dosing, with water allowed. Volunteers remained upright during the first hour after drug administration and activities are limited as necessary.

2.2.2.10.2. Blood sampling. Blood samples (5 mL) were withdrawn just prior to drug administration and at the following time points: 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h post drug administration. The blood samples were withdrawn in pre-heparinized tubes to guard against coagulation of blood. The collected blood samples were centrifuged at 3000 rpm for 10 min to separate the plasma. The separated plasma was then collected in polyethylene capped tubes and stored at −20 °C till required for analysis.

2.2.2.10.3. Quantification of risperidone concentration in plasma. Plasma samples were analyzed for risperidone adopting a modified sensitive, selective and accurate HPLC method, developed and validated before the study.

Analysis of samples was performed using a Shimadzu HPLC system equipped with spectrofluorimetric detector (HPLC apparatus consisting of isocratic pump LC-10AD and RF-551 spectrofluorimetric detector, all from Shimadzu, Kyoto, Japan. The analytical column was a Nova-Pak C18 HPLC column, 300 mm × 3.19 I.D. mm, particle size 5 μm; Waters Associates, USA). The mobile phase was mixture of acetonitrile and water (70:30 V/V), pH was adjusted to the value of 3 using 0.02 N acetic acid. The flow rate was 1 mL/min. The detection was carried out at 277 nm. A calibration curve was plotted for risperidone in the range of 20–260 ng/mL spiked with propyl paraben sodium as an internal standard.

To 500 μL of plasma samples in a glass centrifuge tube, 500 μL of propyl paraben sodium solution of concentration 100 ng/mL were added as internal standard and 500 μL of acetonitrile. After mixing in vortex mixer for 5 min (Paramix II, Julabo, Germany), the mixture was centrifuged for 10 min at 3000 rpm. The supernatant was filtered using Millipore filter (45 μm). Then 20 μL of supernatant was injected into liquid chromatography.

Concentrations of risperidone in unknown samples were calculated with reference to the prepared calibration curve.

2.2.2.10.4. Determination of the pharmacokinetic parameters. To assess the pharmacokinetic profile of risperidone, its total

amount in the collected plasma was displayed as a function of time, and pharmacokinetic parameters were calculated for the tested formulations (C_{max}, T_{max}, $AUC_{(0-24)}$, $AUC_{(0-\infty)}$, $AUMC_{(0-24)}$, $AUMC_{(0-\infty)}$ and MRT).

2.2.2.10.5. Statistical analysis of the pharmacokinetic parameters. All the results were expressed as mean values ± SD. Statistical analysis for pharmacokinetic parameters were performed by applying two paired t-test. Statistical analysis was performed using SPSS® software, USA.

2.2.2.11. The questionnaire. The questionnaire is a technique of assembling information frequently used in scientific-educational research, both quantitative and qualitative. It allows us to collect in a systematic way the required information depending on the variables of the research; and at the same time the questionnaire helps to specify our purpose of study. Moreover, questionnaires are simple to apply, and they offer diverse criteria for answers that are subsequently quantifiable. These are enough reasons to consider the questionnaire as a principal instrument for our research.

In an attempt to study the side effects of the prepared floating microparticles, named sample 1, and to be compared to the side effects of marketed product (Risperidal® 4 mg), named sample 2, the human volunteers were requested to complete a self-administrated questionnaire. The questionnaire was developed to assess the symptoms associated with the side effects that they had expert after an oral risperidone dosage form administration. Questionnaire included five items: headache, drowsiness, nausea, tiredness and difficulty staying awake during the day. The score range for symptoms severity was between 0 and 3, where, score level of 0 indicates no symptoms, score level of 1 indicates little symptoms, score level of 2 indicates quite a lot symptoms and score level of 3 indicates very much symptoms.

Statistical analysis of the score values of the side effects for each sample was performed using the nonparametric analysis of variance (Kruskal–Wallis test) with significance set at $p < 0.05$.

3. Results and discussion

3.1. Preparation of floating microparticles

Floating microparticles were prepared using emulsion solvent diffusion technique. Microparticles are more beneficial than single-unit systems. More reliable gastric emptying patterns are observed for multiparticulate formulations as compared with single unit formulations, which suffer from “all or none concept.” As the multiple unit particulate systems are distributed freely throughout the gastrointestinal tract, their transport is affected to a lesser extent by the transit time of food compared with single unit formulation [45].

The organic solvents chosen, dichloromethane and ethanol, have low toxicity potential compared to many other solvents and do not have any hazardous effect on the body because they evaporate during the process [46].

3.2. Characterization of risperidone floating microparticles

3.2.1. Buoyancy test of risperidone floating microparticles

Floating microparticles were formulated using Eudragit S100, HPMC (50 cp) as well as glyceryl monostearate, Geleol mono and diglyceride, Gelucire 43/01 pellets, Gelucire 44/14, Gelucire 50/13 pellets or Compritol 888 ATO as lipid carriers to modify drug release.

Glyceryl mono stearate is a mixture of glyceryl ester of fatty acids and is composed of glyceryl monostearate (65%), glyceryl monopalmitate (30%) and glyceryl monomyristate (5%). It has different HLB grades and is widely used as an emulsifier and can be used for the preparation of sustained release dosage forms [47].

Gelucire 43/01 is a highly hydrophobic lipid with an HLB value of 1 and a melting point of 43 °C. The extreme hydrophobicity of Gelucire 43/01 provides release-retarding properties and floating behavior [30]. Gelucire 44/14 is a semi-solid excipient with an HLB value of 14 and a melting point at 44 °C. The hydrophilic property of Gelucire 44/14 is useful in dissolution enhancement as well as in controlled-release formulations [48]. Owing to the extreme hydrophilicity and low density, Gelucire 50/13 may be considered an appropriate carrier for designing a fast release floating drug delivery system [49].

Compritol 888 ATO, chemically known as glyceryl behenate is a waxy material, originally introduced as a lubricant in compressing tablets, which has recently had a wide application as a sustained-release agent. The esterification of glycerol by long chain fatty acid gives them a pronounced hydrophobic character with a low HLB value [50]. Its melting point is approximately 70 °C [51]. This lipid has an amphiphilic character due to the presence of partial acylglycerol. Its hydrophilic lipophilic balance (HLB) is approximately 2 and density value is 0.94 g/cm^3.

In an attempt to study the effect of different concentrations of HPMC on the floating behavior of the microparticles, it was found that the buoyancy percentage of the microparticles decreased significantly ($p < 0.05$) with an increase in HPMC concentration from 0.1 to 0.2 and 0.3 g in floating microparticles except those containing Compritol 888 ATO (F16, F17 & F18).

It was noticed that as Eudragit S100 content increased in the microparticles from 0.7 to 0.8 g (F18 and F17, respectively), the buoyancy percentage value of the floating microparticles significantly ($p < 0.05$) increased from 48.07 to 97.95% at the end of 12 h. Owing to the hydrophobic property of Eudragit S100, the hydration of the matrix decreased thus increase in the buoyancy percentage was noticed.

While, further increase in the Eudragit S100 content from 80 to 90% with decrease in HPMC content (F17 and F16, respectively), showed significant ($p < 0.05$) decrease in the buoyancy percentage value from 97.95 to 26.11%. This could be attributed to that the concentration of HPMC had a significant role in the formation of microspheres (Table 3) [52].

These results were attributable to conversion of less spherical form of the resulted floating microspheres on adding HPMC. In addition, 0.1 N HCl (pH 1.2) can readily penetrate floating microspheres due to the dissolution of HPMC in solution resulted in more porosity matrix with less floating ability [41].

The microparticles buoyancy percentage value increased by using Geleol mono & diglyceride (F4, F5 and F6) instead of glyceryl monostearate (F1, F2 and F3). This could be explained by the lipid carrier HLB values as the buoyancy percentage significantly ($p < 0.05$) decreased by increasing HLB value of the lipid carriers from an HLB value of 3 for Geleol mono & diglyceride to an HLB value of 3.8 for glyceryl monostearate resulted in increasing its hydrophilicity which facilitates penetration of water into the microparticles thus reducing the buoyancy.

In an attempt to study the effect of different Gelucire types as lipid carriers on the floating behavior of the microparticles, floating microparticles were prepared containing Eudragit S100 in combination with different HPMC concentrations using Gelucire 43/01, Gelucire 44/14 or Gelucire 50/13.

The obtained results showed that buoyancy percentage values were found to follow the following order, floating microparticles containing Gelucire 43/01 (F7, F8 and F9) > Gelucire 50/13 (F13, F14 and F15) > Gelucire 44/14 (F10, F11 and F12) as shown in Table 3. This could be explained by the HLB values of the Gelucire; buoyancy percentage decreased by increasing the Gelucire HLB value.

Table 3
Results of buoyancy percent, yield percent, drug loading and *in-vitro* drug release of risperidone from floating microparticles.

Formula code	Buoyancy percentage after 12 h[a]	Yield (%)[a]	Drug loading (%)[a]	Release efficiency (RE%)[a]
F1	20.77 ± 2.07	81.50 ± 0.09	7.619 ± 2.03	81.94 ± 2.24
F2	16.38 ± 2.08	73.25 ± 1.03	8.103 ± 1.00	88.34 ± 2.18
F3	13.29 ± 0.07	64.92 ± 1.97	8.016 ± 0.23	93.30 ± 1.82
F4	56.59 ± 4.01	83.28 ± 2.69	6.802 ± 1.52	72.61 ± 3.35
F5	48.30 ± 0.07	82.38 ± 0.54	7.111 ± 1.39	79.76 ± 0.95
F6	35.36 ± 1.37	77.50 ± 0.99	7.135 ± 3.07	86.65 ± 2.09
F7	47.49 ± 3.10	83.19 ± 1.11	6.611 ± 1.99	52.14 ± 6.59
F8	37.10 ± 0.36	79.58 ± 2.67	6.833 ± 0.46	76.72 ± 2.10
F9	23.06 ± 0.98	75.02 ± 0.06	7.683 ± 0.73	81.52 ± 1.92
F10	18.13 ± 1.78	74.95 ± 3.97	7.619 ± 2.12	85.70 ± 0.11
F11	13.91 ± 3.85	65.08 ± 2.41	9.048 ± 0.07	90.42 ± 2.08
F12	11.12 ± 1.42	62.52 ± 2.60	10.317 ± 3.12	92.55 ± 0.02
F13	36.52 ± 1.29	79.10 ± 1.30	7.246 ± 0.71	84.75 ± 1.43
F14	27.16 ± 4.58	72.16 ± 2.13	7.341 ± 1.33	90.59 ± 1.14
F15	15.40 ± 4.03	62.54 ± 1.79	10.151 ± 2.66	90.74 ± 2.21
F16	26.11 ± 4.33	85.02 ± 2.31	8.206 ± 0.77	61.52 ± 1.34
F17	97.95 ± 0.43	82.21 ± 0.67	11.270 ± 1.63	57.52 ± 2.06
F18	48.07 ± 1.85	81.46 ± 0.33	10.071 ± 1.02	83.00 ± 3.20

[a] Each value represents the mean ± SD (n = 3).

Gelucire 43/01 is a highly hydrophobic lipid with an HLB value of 1 and melting point of 43 °C. The extreme hydrophobicity of Gelucire 43/01 provides good floating behavior [30], in comparison with Gelucire 50/13 and Gelucire 44/14 which have HLB values of 13 and 14, respectively. The hydrophilic property of Gelucire 50/13 and Gelucire 44/14 decreased the floating ability of microparticles formulated with these lipids. Also, it was observed that the floating percentage values of floating microparticles containing Gelucire 50/13 are higher than those containing Gelucire 44/14. This may be due to longer carbon chain of Gelucire 50/13.

3.2.2. Percent yield of risperidone floating microparticles

During the preparation of microparticles, the mechanical variables cause loss of final product and hence process yield may not be 100%. Hollow microparticles were weighed after drying and the percentage yield of floating microparticles were calculated to evaluate the microparticles preparation method. Percentage yield values of the microparticles were found to be in the range of 62.52–85.02% in all the formulations (Table 3). The high percentage yield of microparticles indicates the efficacy of the preparation method used. The high yield value may be attributed to the presence of HPMC or Eudragit S100 which yielded uniform dispersion in dichloromethane/ethanol mixture [53].

Increasing HPMC amount from 0.1 to 0.2 and 0.3 g in all prepared microparticles using different lipid carriers (glyceryl mono stearate, Geleol mono & di glycerides, Gelucire 43/01, Gelucire 44/14, Gelucire 50/13 and Compritol 888 ATO), resulted generally in a significant decrease ($p < 0.05$) in percentage yield value of the microparticles. The decreased microparticles yield with increased concentration of HPMC may be the result of flocculation and aggregation of microparticles due to increased viscosity of the preparation by increasing HPMC amount [54]. Nepal et al. [55] have found that increasing polymer concentration led to an increase in yield of microparticles till an optimum value after which the yield decreased.

It was found that the yield values of microparticles depend on the HLB value of lipid carrier used as using Gelucire 44/14 (F10, F11 & F12) and Gelucire 50/13 (F13, F14 & F15) with high HLB values resulted generally in a low yield microparticles compared to the counterparts microparticles formulated with other investigated lipids. This could be attributed to that using lipid carriers with high HLB values can be more miscible with the aqueous phase and resulted in less uniform polymer shell which led to small irregularly shaped microparticles (Fig. 1). While using lipid carriers of low HLB with high lipophilic nature which enhance the ability to form microparticles resulted in high yield values of microparticles.

It was observed that the best yield value was obtained for the microparticles using Compritol 888 ATO as a lipid carrier with yield value of 85.02, 82.21, and 81.46% for F16, F17 and F18, respectively.

3.2.3. Drug loading in floating microparticles

Drug loading during the preparation and subsequent release after administration are two important properties that have to build into a drug delivery system. Drug loading is dependent on the process of preparation, formulation variables, physicochemical properties of the matrix, physicochemical properties of the drug and the interaction between matrix, drug and the surrounding medium.

Table 3 shows the percentage of drug loading of microparticles. Values of drug loading were ranged between 6.611 and 11.270%.

Little increase in drug loading percent was generally observed with increasing HPMC amount from 0.1 to 0.2 and 0.3 g. Drug loading of formulated microparticles was a function of process variables as well as the physicochemical properties of drug. It was observed that variation in polymer concentration influenced the drug content. This could be attributed to that the increase of the viscosity at higher polymer concentration restricted the movement of drug from polymer matrix into aqueous phase.

Fig. 1. Image showing irregular shape of risperidone floating microparticles containing Gelucire 44/14 (F10, F11 & F12) and Gelucire 50/13 as lipid carriers (F13, F14 & F15).

3.2.4. In-vitro drug release studies from floating microparticles

Evaluation of the *in-vitro* drug release is considered an important step during the development and quality control of a new dosage form. Although the *in-vivo* testing is essential in the development and evaluation of dosage forms, assessment of *in-vitro* characteristics and quality of the product is also necessary.

In an attempt to study the effect of HLB value of the lipid carriers on the drug release of floating microparticles, microparticles containing Eudragit S100 in combination with different HPMC concentrations with glyceryl mono stearate, Geleol mono and diglyceride, Gelucire 43/01, Gelucire 44/14, Gelucire 50/13 and Compritol 888 ATO as lipid release modifiers were subjected to this study.

It was noticed that increasing HPMC content from 0.1 to 0.2 and 0.3 g in floating microparticles (F1, F2 and F3 or F4, F5 and F6 or F7, F8 and F9 or F10, F11 and F12 or F13, F14 and F15 or F16 and F18, respectively), resulted in fast release efficiency values ($p < 0.05$). This could be due to the high permeability and hydrophilic nature of HPMC which increases the porosity of matrix and accelerates the drug release [56].

It was observed that as the HLB value of the lipid release modifier decreased from HLB value of 3.8 for glyceryl monostearate (F1, F2 & F3) to 3 for Geleol mono and diglyceride (F4, F5 & F6) in the prepared microparticles, the RE of the drug from the floating microparticles decreased ($p < 0.05$) (Table 3). This higher extent of drug release in case of microparticles prepared by glyceryl mono stearate could be attributed to the surface-active property of this lipid (HLB value 3.8) [57]. An increase in water solubility of surfactant mixtures could produce a microporous matrix within the microparticles for water diffusion and increases the rate of drug dissolution and diffusion to the release medium [58].

It was found that the RE values of floating microparticles containing Gelucire 43/01 (F7, F8 and F9) are less than those containing Gelucire 44/14 (F10, F11 and F12) or Gelucire 50/13 (F13, F14 and F15) (Table 2). This may be attributed to that Gelucire 43/01 is a highly hydrophobic lipid with an HLB value of 1 and a melting point of 43 °C. This extreme hydrophobicity of Gelucire 43/01 provides release retarding properties and floating behavior [30].

However, there was no significant difference ($p > 0.05$) in release efficiency of risperidone from floating microparticles containing Gelucire 50/13 (F13, F14 and F15) and those containing Gelucire 44/14 (F10, F11 and F12).

It was observed that the RE values of floating microparticles containing Compritol 888 ATO (HLB = 2) as lipid carrier (F16, F17 and F18), were significantly less ($p < 0.05$) than those containing Gelucire 44/14, Gelucire 50/13, Geleol mono & diglyceride or glyceryl monostearate (Table 3). This may be attributed to that Compritol 888 ATO decreases the hydration of microparticles matrix and retards the drug release by erosion mechanism owing to its hydrophobic property [59].

Mostly the initial burst release of risperidone from floating microparticles containing Gelucire 44/14 or Compritol was low compared to those containing other lipid carriers. The initial burst drug release in all the formulation followed the order F7, F8 and F9 < F16, F17 and F18 < F4, F5 and F6 < F1, F2 and F3 < F13 and F14 < F12 < F10 and F11 for microparticles containing the lipid part Gelucire 43/01 (HLB = 1), Compritol 888 ATO (HLB = 2), Geleol (HLB = 3), glyceryl mono stearate (HLB = 3.8), Gelucire 50/13 (HLB = 13) and Gelucire 44/14 (HLB = 14), respectively during the first 30 min. This order was found to be attributed to the HLB value of the lipid part of the microparticles as when the lipid HLB value was increased, the drug release increased (Figs. 2–4).

The initial burst release of the drug from the system is often therapeutically undesirable because the total amount of drug released is remarkably influenced by this initial control of release

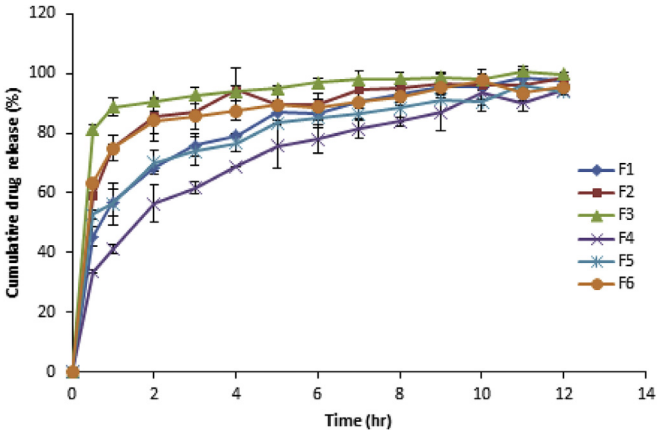

Fig. 2. Release profile of risperidone from floating microparticles F1-F6 at pH 1.2.

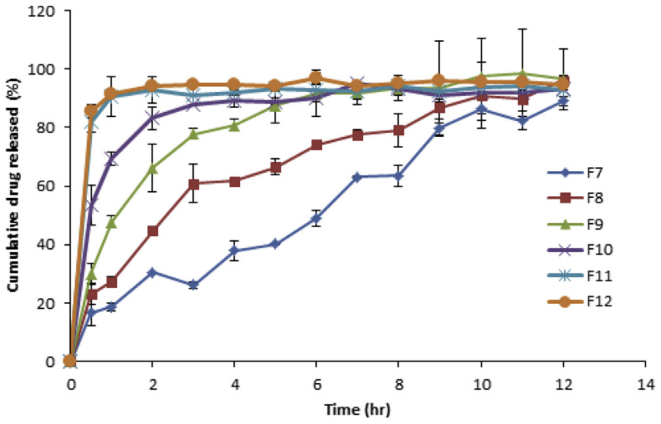

Fig. 3. Release profile of risperidone from floating microparticles F7-F12 at pH 1.2.

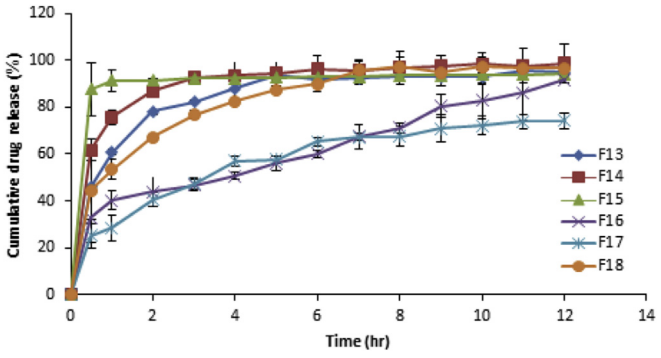

Fig. 4. Release profile of risperidone from floating microparticles F13-F18 at pH 1.2.

from the dosage form [60]. Therefore, the initial burst release is one of the major challenges in developing sustained release microparticle systems. The release of a large bolus of drugs prior microparticles reach a steady state release is both therapeutically undesirable and economically ineffective. Hence, the ability to control and limit the initial burst release is highly sought-after and extensively studied.

The large initial release can be attributed to both surface associated drug as well as the porous structure of microparticles. The porous structure facilitated diffusion of water into the microparticle as well as drug out from the microparticle by acting as transport

pathways for drug and water molecules [61]. Microparticles with more pores tend to show a more rapid drug release [62].

Generally, it was observed that by increasing the amount of Eudragit S100 with decreasing amount of HPMC in the microparticles, the initial burst release significantly decreased ($p < 0.05$). This may be attributed to decreasing hydrophilicity of the matrix inhibiting water uptake from the release medium, which in turn resulted in lower initial burst release accompanied with a decrease in the porosity of the matrix [63].

Among all formulations studied it was found that as, increasing the amount of Eudragit S100 in the microparticles from 0.7 to 0.8 & 0.9 g, resulted in a decrease in the percent of risperidone released ($p < 0.05$). The increase in Eudragit S100 in the microparticles leads to the increased density of polymer matrix in the microparticles which results in an increased diffusional path length. This may decrease the overall drug release from polymer matrix [64].

Floating microparticles F17 containing Compritol 888 ATO as a lipid carrier and 80% Eudragit S100 represents the best release action on the release of the drug amongst the formulations as 25.12% of drug released after 30 min and it had t_{50} and t_{90} values of 5.265 and 8.677 h, respectively. Furthermore, this formula showed excellent floating behavior as its buoyancy percentage value was 97.95% at the end of 12 h.

Hence, formula F17 was subjected to further modifications (Table 2) in order to decrease the burst effect taking into consideration the objective of this work, achieving a compromise between controlled drug release characteristics and excellent floating behavior.

3.3. Preparation of modified floating microparticles of risperidone

The modified floating microparticles of risperidone were prepared by emulsion solvent diffusion technique as previously mentioned. Then the obtained microparticles were subjected to various characterizations.

3.4. Characterization of modified floating microparticles of risperidone

3.4.1. Buoyancy test of modified floating microparticles

The buoyancy percentages of the microparticles prepared using different drug loading were investigated as shown in Table 4. It was found that by increasing drug loading in the prepared microparticles from 0.1 to 0.125, 0.150, 0.200 and 0.300 g (F17, F19, F20, F21 and F22, respectively), the buoyancy percentage value significantly ($p < 0.05$) decreased. This finding might be attributed to that upon increasing drug loading, the uniform polymer shell of microparticles was unable to form, a large number of needle like shape crystals of drug can be generated that facilitated the penetration of release medium into the microparticles through the porous surface, thus reducing the buoyancy [65].

Thus, the optimum loading amount of risperidone appeared to be 0.100 g, as at that point, buoyancy of the microparticles attained its highest level.

In an attempt to study the effect of various viscosity grades of HPMC on the microparticles floating behavior, microparticles were formulated with various viscosity grades of hydroxypropylmethyl cellulose (HPMC); HPMC E50, HPMC E15, HPMC E6 and HPMC E4 (F17, F23, F24 and F25, respectively).

Table 4 illustrates the floating behavior of floating microparticles containing various grades of HPMC. The buoyancy percentage values of the prepared microparticles F17, F23, F24 and F25 were found to increase with an increase in the HPMC viscosity (97.25, 38.54, 32.15 and 31.79%, respectively). F17 containing HPMC E50 having high viscosity forms a strong barrier to the ingress of release medium, hence have higher percentage of buoyancies than those containing lower viscosities of HPMC.

Cyclodextrins have been used in pharmaceutical formulations in order to enhance the solubility, dissolution rate, stability, bioavailability, and oral absorption or to modulate biological activity of drugs [66–68]. Moreover, these substances are also used to modify the drug release from different systems: The hydrophilic CD derivatives such as 2-hydroxylpropyl-β-cyclodextrin (2-HPβCD), randomly methylated-β-cyclodextrin (MβCD), dimethyl-β-cyclodextrin (DMβCD) and sulfobutyl ether β-cyclodextrin (SBEβCD) are used for improving solubility and dissolution rate of poorly water-soluble drugs [69], while hydrophobic CD derivatives such as ethylated and acylated CD are used as slow-release carriers for water-soluble drugs [70]. Furthermore, advanced controlled-release profiles can be achieved by a combination of CD derivatives and pharmaceutical additives [71].

In attempt to evaluate the influence of cyclodextrins on the floating performance of the prepared microparticles, floating microparticles of risperidone were formulated using different ratios of different types of cyclodextrins as a complexing agent.

It was observed that the buoyancy percentage values of microparticles containing methyl-β-cyclodextrin are higher than those containing hydroxypropyl-β-cyclodextrin, which may be due to the hydrophilicity of cyclodextrins as hydroxypropyl-β-cyclodextrin is more hydrophilic than methyl-β-cyclodextrin [72]. Also, it may be due to the molecular weight of methyl-β-cyclodextrin being less than the molecular weight of hydroxypropyl-beta-cyclodextrin which resulted in less dense microparticles.

The buoyancy percentage values of F26 and F27 microparticles containing 1:1 and 1:2 M ratio of drug to hydroxypropyl-β-cyclodextrin were significantly decreased ($p < 0.05$) to 60.31 and 64.46%,

Table 4
Results of buoyancy percent, yield percent, drug loading of and *in-vitro* drug release of risperidone from modified floating microparticles.

Formula code	Buoyancy percentage after 12 h[a]	Yield (%)[a]	Drug loading (%)[a]	Release efficiency (RE%)[a]
F19	53.34 ± 1.21	73.98 ± 2.15	10.357 ± 0.98	86.44 ± 1.29
F20	42.11 ± 3.09	71.63 ± 1.03	9.444 ± 0.12	88.34 ± 0.94
F21	32.09 ± 2.23	67.77 ± 0.12	9.048 ± 1.66	92.91 ± 0.26
F22	28.75 ± 2.11	61.01 ± 0.99	7.698 ± 1.84	82.50 ± 4.08
F23	38.54 ± 3.65	79.90 ± 2.09	9.595 ± 2.08	70.68 ± 0.21
F24	32.15 ± 1.09	77.31 ± 1.22	8.952 ± 0.23	73.81 ± 1.09
F25	31.79 ± 3.10	78.54 ± 1.38	7.897 ± 3.88	84.65 ± 1.52
F26	60.31 ± 0.85	73.21 ± 0.79	5.905 ± 1.85	87.48 ± 3.03
F27	64.46 ± 0.59	76.92 ± 0.31	9.500 ± 1.67	85.44 ± 0.18
F28	93.13 ± 0.54	72.64 ± 3.01	9.325 ± 0.98	74.64 ± 0.35
F29	89.88 ± 2.39	71.99 ± 1.55	9.683 ± 0.61	86.80 ± 2.01
F30	91.20 ± 3.74	76.11 ± 2.87	10.875 ± 1.22	75.93 ± 1.39
F31	95.93 ± 1.99	79.73 ± 3.14	12.540 ± 0.10	82.41 ± 3.87

[a] Each value represents the mean ± SD (n = 3).

respectively compared to their counterpart particles without cyclodextrin (F21) (Table 4).

The buoyancy percentage values of F28, F29, F30 and F31 microparticles containing 1:1, 1:2, 1:3 and 1:4 M ratio of drug to methyl-β-cyclodextrin ratio were 93.13, 89.88, 91.20 and 95.93%, respectively as shown in Table 4.

It was found that F31 exhibited excellent floating ability which persisted for 95.93% 12 h.

3.4.2. Percent yield of modified floating microparticles

The production yield for the modified microparticles was decreased by increasing the initial drug load from 0.100 to 0.125, 0.150, 0.200 and 0.300 g in microparticles F17, F19, F20, F21 and F22, respectively. This could be attributed to that upon increasing the loading amount of risperidone, the uniform polymer shell of microparticles might not be able to be formed and a large amount of fiber-like precipitate was obtained. Also, the entrapment of a large amount of undissolved drug in the polymer shell might lead to decrease rigidity of the shell and hence aggregation of premature microparticles into large clumps [73].

The yield values of further modified microparticles F19–F31 were found to be in the range of 61.01–79.73% as shown in Table 4. The low percentage yield in some formulations may be due to microparticles lost during the washing process. The best yield was obtained in F31 with yield value of 79.73% which indicates optimum diffusion of solvents.

3.4.3. Drug loading in modified microparticles

Table 4 illustrates the drug loading values of microparticles containing different release modifiers. The values lie between 5.905 and 12.540%.

It was noticed that the values of drug loading was decreased by increasing the amount of initial drug load from 0.100 to 0.125, 0.150, 0.200 and 0.300 mg in microparticles F17, F19, F20, F21 and F22, respectively as shown in Table 4.

This could be due to the high initial drug load with respect to the polymer amount. This might lead to two results. First, the microparticles with too much suspended drug were more irregular in shape and had more porous structure. The highly porous surface permitted more penetration of the aqueous phase and hence more drug extraction out of the microparticles. Secondly, too much drug load increased the risk of drug leakage due to the limited space inside the microparticles and the shrinkage of the microparticles during its solidification [74].

It was found that drug loading values of microparticles containing HPMC polymers (F23, F24 & F25) have been increased with an increase in the viscosity of HPMC in the formulation (Table 4). This may be due to an increase of drug content in the swollen or gel structure of HPMC.

3.4.4. In-vitro drug release studies from modified floating microparticles

Table 4 reveals that by increasing drug loading in the floating microparticles from 0.100 g to 0.125, 0.150 and 0.200 g (F17, F19, F20 and F21, respectively), the RE% increased significantly ($p < 0.05$). Also, it was observed that the burst drug release was increased with increasing drug loading due to the elution of surface associated drugs and high concentration gradient between microparticle and surrounding release medium [75–77]. Also, it was observed that increasing in drug loading leads to significant decrease in buoyancy percentage and yield values (Table 4). Further increase in drug loading in microparticles from 0.200 to 0.300 g (F21 and F22, respectively), leads to a decrease in the drug release from the microparticles. The slow drug release can be explained by the agglomeration of the lipophilic drug in the microparticle matrix that acted as a barrier for the penetration medium, thereby, retarding the diffusion of drug.

The present investigation applied on the floating microparticles of risperidone with various grades of polymer hydroxypropylmethyl cellulose (HPMC); HPMC E50, HPMC E15, HPMC E6 and HPMC E4 (F17, F23, F24 and F25, respectively).

From the release profiles, it was observed that the variation in grade of polymer had variable effect on drug release (Figs. 4–6). The *in-vitro* release of risperidone from different formulations showed that the RE values of the formulations were more or less comparable (Table 4), it was found to follow the following order F25 > F24 > F23 > F17. A decrease in the amount of drug released was observed with an increase in the viscosity grades of the polymer. This slow release could be attributed to the formation of a thick gel structure that delays drug release from the matrix. In other words, HPMC E4 with low viscosity tended to attribute for quick release of the drug and HPMC E50 with high viscosity retarded the release of the drug.

The *in-vitro* drug release studies indicated that the floating microparticles containing HPMC E50 with high viscosity grade (F17) showed small burst effect in comparison to other batches. The results indicate that these floating microparticles provide a better option for controlled initial drug release but it has slow drug release over 12 h and thus it needs further modifications.

Fig. 7 illustrates the release profile of risperidone from floating microparticles containing cyclodextrins as a modified release carriers to modify the drug release site and/or time profile. CDs seem to be the most important ones in this domain. Also, the incorporation of CDs into a drug dosage form may affect the activity of basic pharmaceutical ingredients and may modify the properties of the whole drug formulation [78].

In an attempt to improve the release of risperidone from floating microparticles, hydroxypropyl-beta-cyclodextrin (HPβCD) or methyl beta cyclodextrin (MβCD) were used as a complexing agent to improve the drug solubility and bioavailability. Therefore, microparticles F26 and F27 were formulated using HPβCD of ratios 1:1 and 1:2, drug to cyclodextrin, respectively. Microparticles F28 and F29 were formulated using MβCD of M ratios 1:1, 1:2, drug to cyclodextrin, respectively.

The comparative results of release studies of risperidone microparticles in presence of cyclodextrins showed increase of drug release compared to those microparticles formulated without the presence of cyclodextrins (F17).

F26 and F27 containing hydroxypropyl-beta-cyclodextrin showed high initial burst effect. As it was observed that about of 65.35 and 61.90% of the drug were released after 30 min from

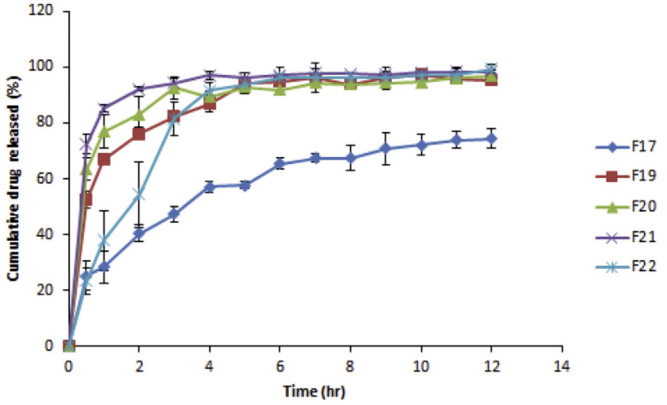

Fig. 5. Release profile of risperidone from floating microparticles F17, F19-F22 at pH 1.2.

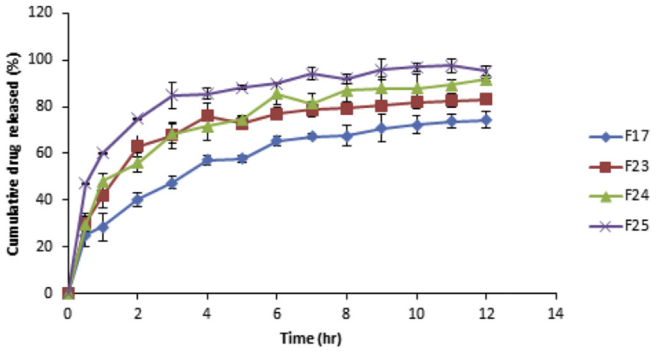

Fig. 6. Release profile of risperidone from floating microparticles F17, F23-F25 at pH 1.2.

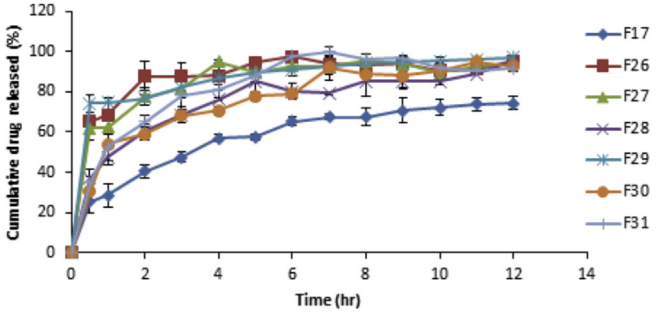

Fig. 7. Release profile of risperidone from floating microparticles F17, F26-F31 at pH 1.2.

formulations containing HPβCD (F26 and F27, respectively). This may be due to the hydrophilicity of hydroxypropyl-beta-cyclodextrin.

It was noticed that increasing the amount of HPβCD from 1:1 to 1:2 M ratio of drug to HPβCD (F26 and F27, respectively) resulted in non-significant increase in drug release efficiency ($p > 0.05$) (Table 4). While increasing the amount of MβCD from 1:1 to 1:2 (F28 and F29, respectively) resulted in significant increase in drug release efficiency ($p < 0.05$), so more drug to MβCD ratios were formulated (Table 4).

The increase in the release of risperidone with increasing MβCD concentration in the microparticles could be explained by the principal of hydrophilicity, inclusion complex formation and the amorphous form generation of risperidone. The high solubility of MβCD in water resulted in better wettability of drug particles and local enhancement of its solubility at the diffusion layer surrounding the drug particles. Subsequently, the interaction between the hydrophobic drug molecule and hydrophobic cavity of MβCD resulted in the formation of an inclusion complex [79].

While further increase in the molar ratio of MβCD to the drug in the prepared microparticles from 1:2 to 1:3 and 1:4 in F29, F30 and F31, respectively, showed decrease in drug release. This can be explained that MβCD improves the total amount of drug released up to a threshold loading concentration, after which further addition of MβCD decreased the amount of drug released because high loading concentration of MβCD results in a favorable arrangement of CDs within the polymer network leading to reduced binding of drug to the CDs [80].

Generally, the microparticles containing cyclodextrin showed good floating behavior. While formula F31 exhibited the targeted modified drug release with moderate burst effect and high drug release efficiency in 12 h as well as instantaneous and excellent floating ability which persisted 95.93% for 12 h. Therefore it was chosen for further investigations.

3.4.5. *In-vitro release study of marketed Risperidal® tablet (4 mg)*

Fig. 11 illustrate the release profile of risperidone from marketed Risperidal® tablet (4 mg). Results show immediate release of risperidone which expected to have large in-vivo C_{max} value resulting in extrapyramidal side effects. On the other hand, F31 shows gradual drug release over 12 h and thus it is expected that such formula would give sustained in-vivo drug release with low Cmax value.

3.4.6. *Scanning electron microscopy (SEM)*

Morphology, surface topography and internal cross-sectional structure of the floating microparticles were investigated using scanning electron microscope (Fig. 8).

SEM photos indicate that the prepared microparticles are generally spherical with smooth surfaces (Fig. 8). Presence of hollow cavity in the microparticles is due to collapse of the wall of the microparticles during the *in-situ* drying process (Fig. 8). Thus, microparticles floated more than 12 h because of presence of hollow cavity [81] that may be also caused by evaporation of solvent entrapped within the shell of microparticle after forming smooth and dense layer. Fig. 9 reveals distinct pores in the microparticle internal structure; these can influence drug release. Porosity of the microparticles can be due to rapid escape of the volatile solvents during formation [46].

Transitional sections for the investigated microparticles revealed that the spherical central hollow core of the microparticles is surrounded by a thick shell with 27.72 ± 3.55 μm thickness

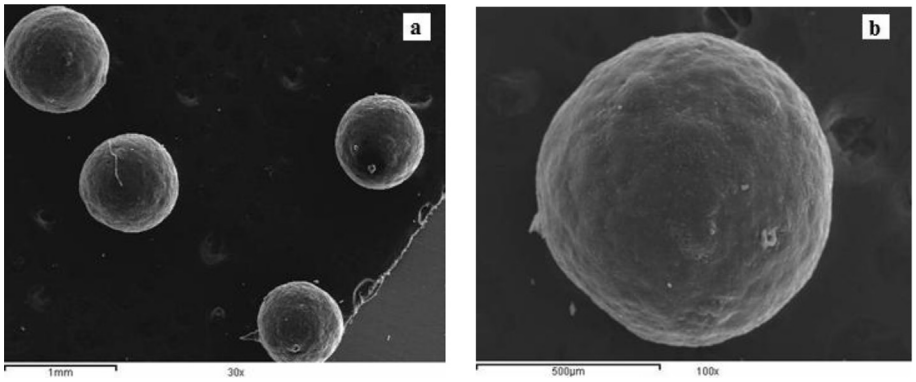

Fig. 8. Scanning electron microscopy photos (SEM) of microparticles F31. (a) entire microparticles, (b) cross section of microparticles.

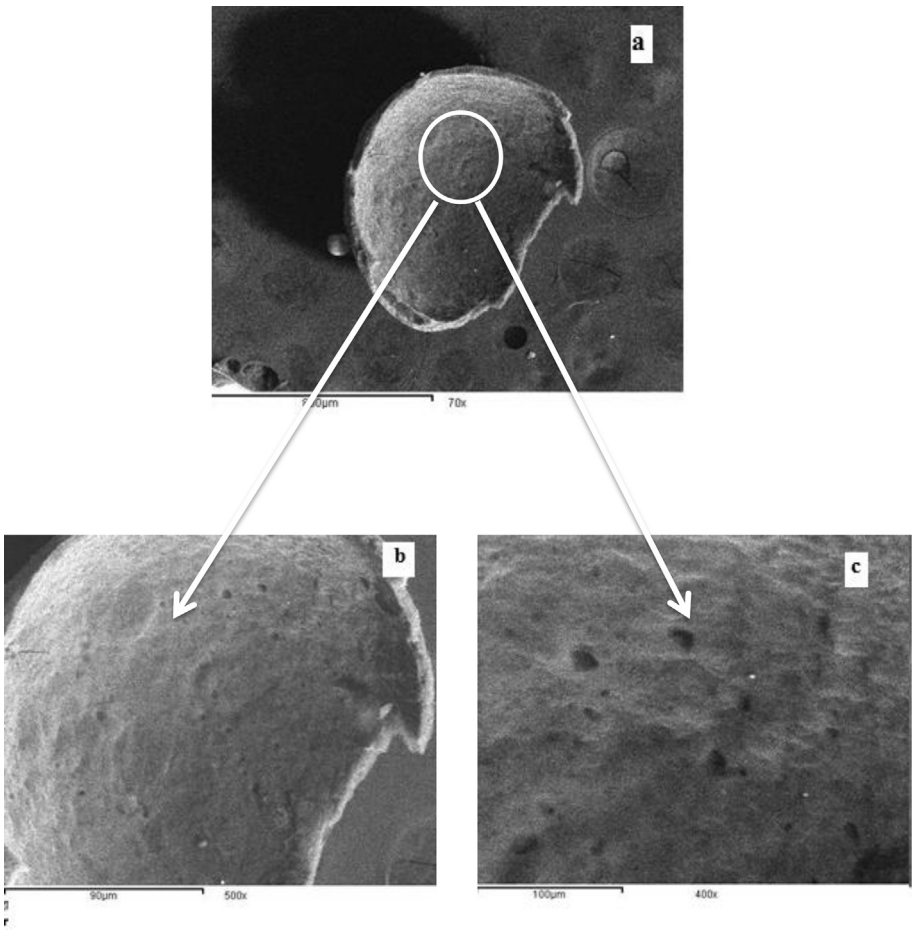

Fig. 9. Scanning electron microscopy photos (SEM) of inner walls of microparticles F31. (a) View of inner cavity, (b) and (c) internal cavity structure with pores.

(Fig. 10). While the particle size of microparticles ranged between 743 and 1100 μm.

3.4.7. Differential scanning calorimetry (DSC)

Differential scanning calorimetry has been very effective in determining the physiochemical properties of different pharmaceutical products, thus facilitating design of new drugs or improving modifications of the existing compounds [82]. It is very important to establish the existence of any incompatibilities during the preformulation stage to ensure the success of the subsequent studies [83].

A DSC curve provides thermal parameters, including the melting point, melting enthalpy, glass transition, crystallization and decomposition temperatures of the active pharmaceutical ingredient. In addition, interactions are evaluated according to the appearance, shift or disappearance of endothermic or exothermic peaks and variations in the corresponding enthalpy values in the thermal curves of the drug–excipient mixtures [84–86].

In order to determine the physical state of drug, i.e., whether amorphous or crystalline, before and after floating microsphere formulation as well as to find the presence of any interaction among the drug and the excipients, DSC examination was conducted for the pure drug, the polymer and physical mixture. The polymorphic structure of a drug is an important parameter which influences the dissolution rate and bioavailability of drug [87].

The DSC curves of the pure drug and the pure polymers Eudragit S100, HPMC, Compritol 888 ATO and methyl-β-cyclodextrin as well as their corresponding physical mixtures are shown in Fig. 12. The DSC curve of the pure drug shows a sharp endothermic peak at 170 °C, corresponding to its melting point and indicating its crystalline nature.

The thermal curve of Eudragit S100 shows endothermic effect at 377 °C attributed to the melting of its crystalline portion.

The DSC thermogram of HPMC shows no sharp endothermic peaks indicating no exact melting points. A broad endothermic bend in the thermogram appears from 40 to 110 °C, which might be due to the volatilization of adsorbed water.

The DSC thermogram of Compritol 888 ATO shows a sharp endothermic peak at 71 °C, due to its crystalline nature.

The DSC thermogram of pure methyl-β-cyclodextrin shows a broad endothermic peak between 40 and 120 °C, which corresponds to water loss. Also, it shows an endothermic effect around 320 °C, which is corresponding to the cyclodextrin melting point.

Fig. 12 shows decrease in the melting endotherm of drug in the physical mixture which could be due to the mixing of drug and excipient, which lowers the purity of each component in the mixture.

DSC thermogram of F17 showed complete disappearance of the characteristic endothermic peak of the drug at 170 °C, indicating that the drug was completely dissolved within the carrier in the formulation. This may indicate the formation of a solid solution (one phase) of the drug within the formula. Also, indicating the conversion of crystalline nature of risperidone to amorphous form.

The DCS thermogram of microparticles F31 shows the disappearance of the drug melting endothermic peak which may be attributed to the inclusion of the drug in the cyclodextrin cavity,

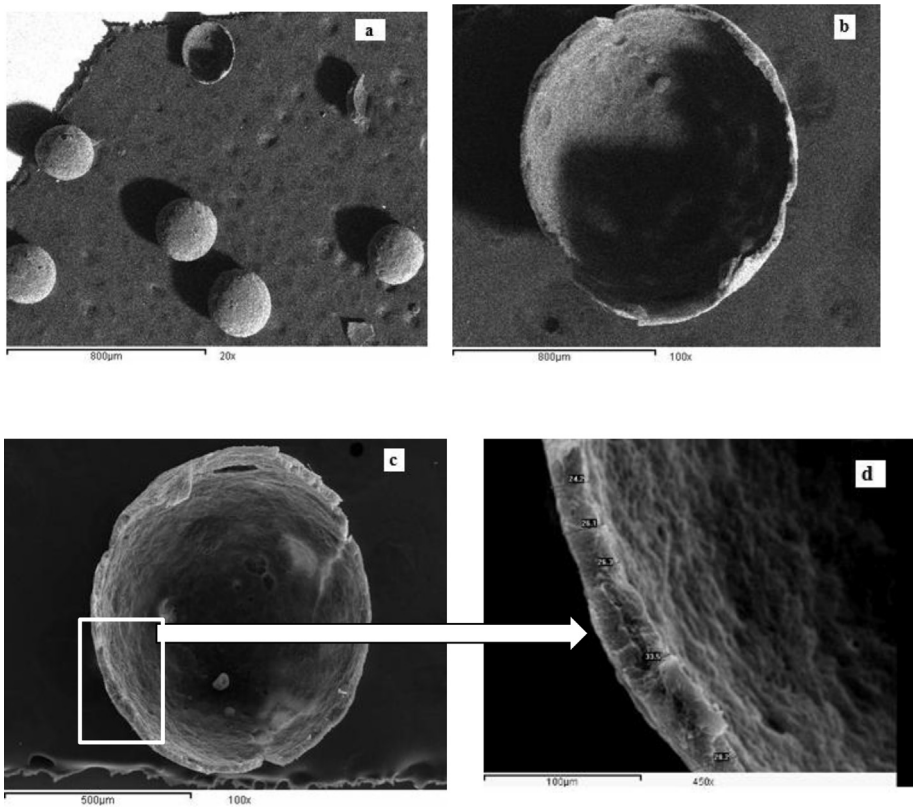

Fig. 10. Scanning electron microscopy photos (SEM) of cross section microparticles F31. (a) and (b) Cross section of microparticles, (c) structure of microparticles walls, (d) thickness of cavity walls.

suggesting the formation of inclusion complex. While presence of the broad endotherm at 72.17 °C might be due to water loss.

As a matter of fact, drug crystallization could lead to thermodynamically unstable system during storage. The growth of drug crystals in the formulation leads to reduction of the drug thermodynamic activity and this consequently may reduce drug release and stability. Amorphization of the drug is thus desirable as the main expertise of formulation development for significant improvement of its bioavailability [88].

X-ray diffraction studies were performed to further investigate the solid state of risperidone.

3.4.8. *X-ray diffraction studies (XRD)*

In order to characterize the physical state of the drug whether amorphous or crystalline before and after formulating it in floating microparticles, x-ray diffraction study was performed. The diffractograms of the pure drug, pure polymers and formulated microparticles (F17 & F31) were performed as well as their corresponding physical mixtures were added for comparison (Fig. 13).

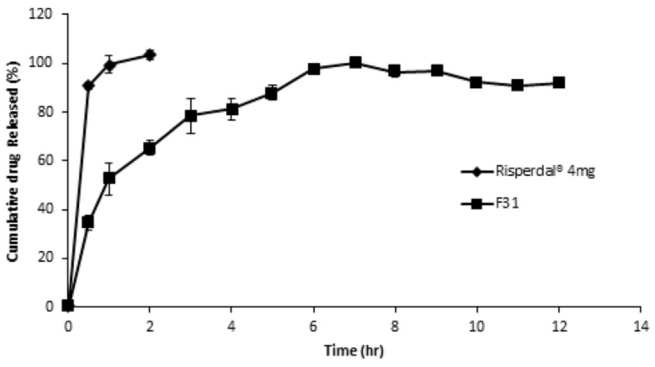

Fig. 11. Release profiles of risperidone from F31 and Risperidal tablet® (4 mg).

The diffraction spectrum of pure risperidone showed that the drug is crystalline in nature as demonstrated by numerous distinctive peaks. The polymorphic structure of a drug is an important parameter that influences the dissolution rate and bioavailability of the drug. The prominent drug peak of high intensity at 2θ value of 21.17° and peaks of lower intensities at 2θ values of 14.05°, 18.73°, 19.63°, 23.03° and 28.87° is in agreement with x-ray data found for risperidone [89].

The diffraction patterns of the investigated polymers are shown in Fig. 13. Eudragit S100 and HPMC diffraction patterns show no characteristic peaks indicating that they are amorphous in nature. Compritol 888 ATO shows two peaks that occur due to lipidic polymorphism, the most abundant peak is observed at 2θ value of 21.11° and peak of lower intensity at 2θ value of 23.09°.

The XRD profile of the physical mixtures for F17 and F31 was simply the superimposition of those of the pure components, demonstrated the crystalline peaks for risperidone corresponding to 2θ value of 21.17° and peaks of lower intensities 2θ values at 14.05°, 18.73° and 19.63° as well as the two characteristic peaks for Compritol 888 ATO at 2θ of 21.11° and 23.09°; however, the peaks intensity were lessened. This data lead to the fact that the drug maintained its crystalline form within the lipid carrier in physical mixture of F17 (Fig. 13), which means that the presence of risperidone in the physical mixture has no influence on its physical state.

Investigated floating microparticles F17 and F31, showed completely different x-ray diffraction patterns than its corresponding formulating materials. The principal peaks of the drug appeared lower in intensity which indicates that the drug was incorporated into the polymer matrix with amorphous drug pattern.

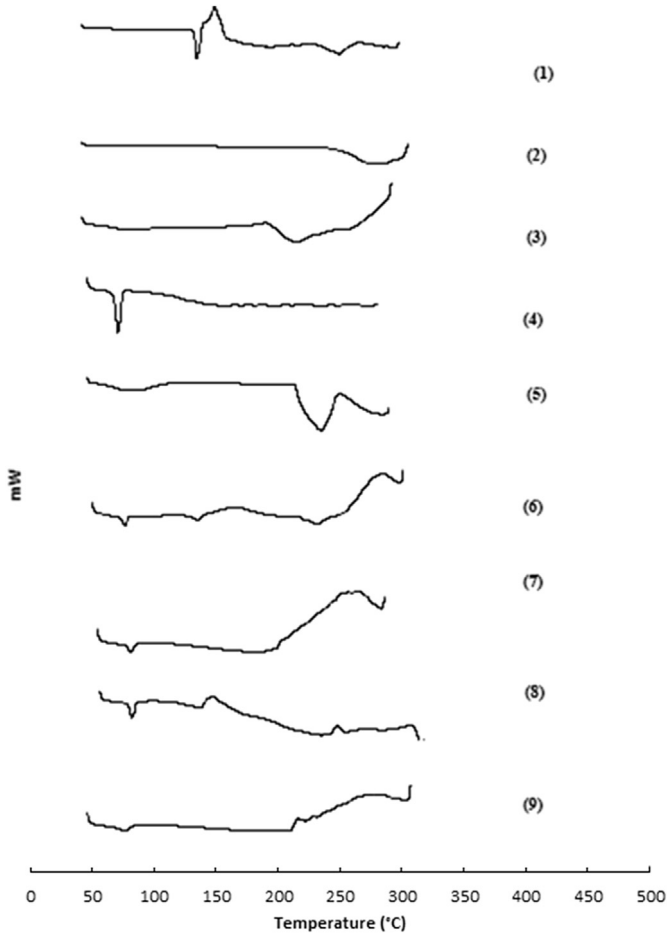

Fig. 12. Differential scanning thermogram of: (1) Risperidone; (2) Eudragit S100; (3) HPMC; (4) Compritol 888 ATO; (5) MβCD (6) physical mixture of F17; (7) F17; (8) physical mixture of F31 and (9) F31.

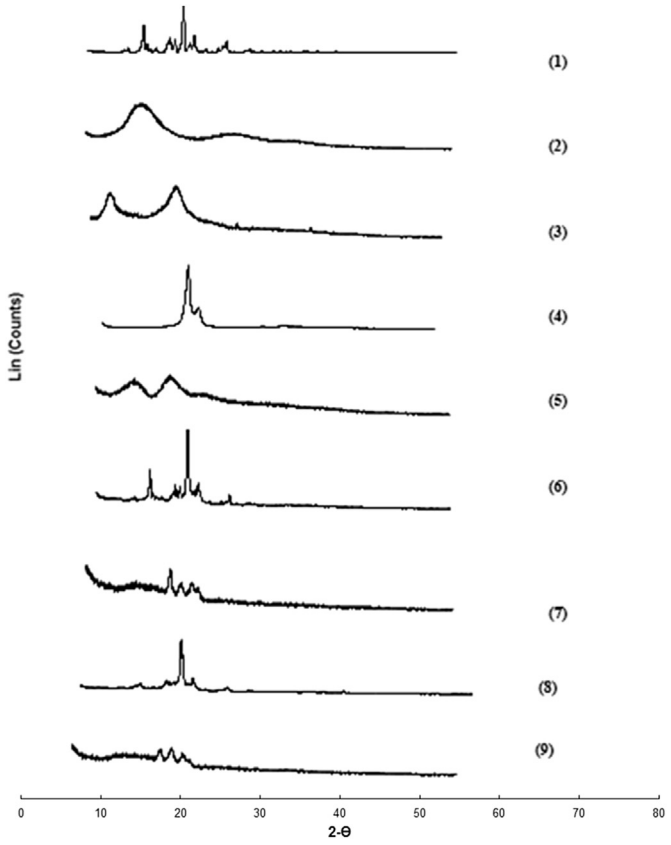

Fig. 13. X-ray diffraction patterns of: (1) Risperidone; (2) Eudragit S100; (3) HPMC; (4) Compritol 888 ATO; (5) MβCD (6) physical mixture of F17; (7) F17; (8) physical mixture of F31 and (9) F31.

The F31 floating microparticles showed lower intensity for drug characteristic peaks than that of F17 floating microparticles. This might be due to the inclusion of the drug in the cyclodextrin cavity, suggesting the formation of an inclusion complex.

3.4.9. In-vivo study of the floating ability of the microparticles

The *in-vivo* floating behavior of the floating F31 microparticles loaded with barium sulphate was investigated by radiographic images (x-ray photographs) of human's stomach at specific periods. The amount of x-ray opaque material (barium sulphate of 1.5% w/w in microparticles) was experimentally determined to allow x-ray visibility but not to hinder microparticles buoyancy [90].

Fig. 14a reveals that the stomach was empty before dosing according to fasting condition (overnight) and to ensure absence of radio opaque materials in the stomach. As presented in Fig. 14b–e, the prepared floating microparticles did not adhere to the gastric mucous and floated on the gastric fluid for more than 12 h and was not removed by the housekeeper wave which was the main challenge against the floating dosage forms.

The floating microparticles appeared as clusters in the gastric area of the human. This was evident by the x-ray photographs taken at 2, 4, 8 & 12 h. After 12 h of dosing the formulation (Fig. 14e), the microparticles appeared faint, distributed along gastric area began to enter the intestine to empty some of the beads (most probably sinking units) from the stomach.

More investigations should be done on human volunteers in order to confirm the results obtained by the *in-vivo* buoyancy study.

3.4.10. Plasma pharmacokinetics of risperidone from the tested formulations

Floating microparticles F31 equivalent to 0.1 g of risperidone and containing HPMC E50, Eudragit S100, Compritol 888 ATO as lipid carrier and MβCD in a M ratio of 1:4 for drug to cyclodextrin was chosen after extensive *in-vitro* investigation, to perform the *in-vivo* studies. The selected formula F31 had the required release profile as it achieved sustained drug release over 12 h. In addition, the formula had excellent *in-vitro* and *in-vivo* floating behavior which extended over 12 h. The formulation (equivalent to 4 mg risperidone) was compared to the marketed product (Risperidal® 4 mg tablet).

Commonly encountered noncompliance to antipsychotic drug therapy leads to an almost double re-hospitalization from relapse. Therefore, a controlled release floating microparticles of risperidone was developed to optimize its blood level, minimize its side effects, and ultimately improve its treatment adherence.

It was revealed that a low C_{max} value was observed for F31 ($p < 0.05$) which was around two times lower than that for Risperidal® tablet (Table 5). The T_{max} values was significantly different between the tested formulations ($p < 0.05$), where F31 had the longer T_{max} value of 4 h, while the Risperidal® tablet had shorter T_{max} value of 3 h. Delayed T_{max} of the F31 indicates that it could successfully achieve more gradual drug release (Fig. 15).

Determination of the area under the curve (AUC) is considered an important parameter for identifying the availability of the drug from the tested formulations. There was significant difference ($p < 0.05$) between the $AUC_{(0-24)}$ or $AUC_{(0-\infty)}$ values of F31 and those for Risperidal® tablet. It was observed that F31 showed higher

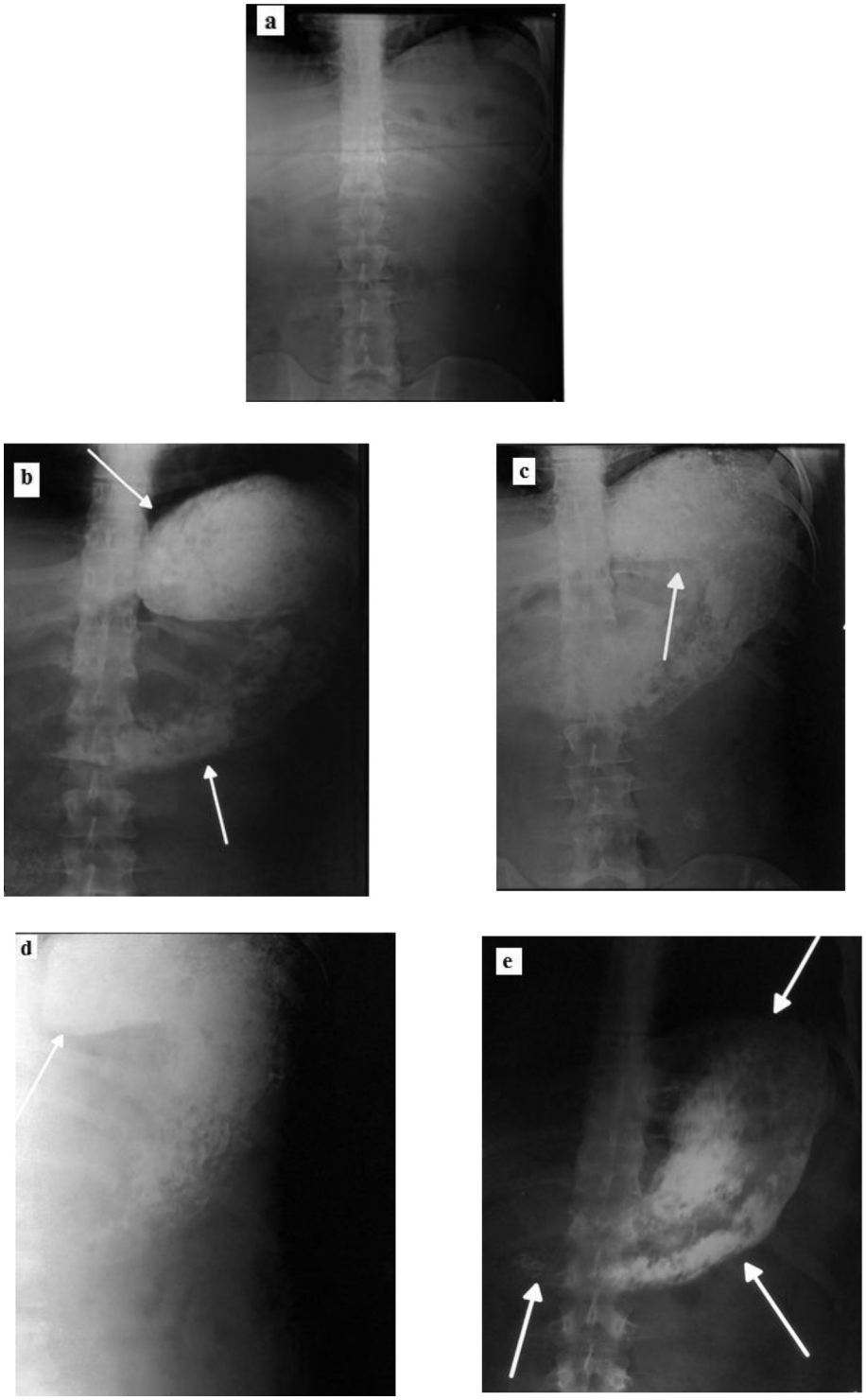

Fig. 14. X-ray photographs showing floating ability of F31 microparticles at different time interval (a) before dosing, (b) 2 h, (c) 4 h, (d) 8 h and (e) 12 h after dosing.

AUC values compared to those for the Risperidal® tablet which indicates better availability of the drug from F31 in comparison to Risperidal® tablet.

Finally, the duration of action for the tested formulations was determined by their mean residence time (MRT) values. There was no significant difference in the MRT values between tested formulations.

Pharmacokinetic study has suggested that the administration of risperidone as floating microparticles would provide controllable and continuous release of risperidone with significant lower C_{max} and higher T_{max} values, compared with the marketed oral product, which allow the probability to optimize the therapeutic window of the drug with fewer extrapyramidal side effects. Furthermore, significant higher AUC value indicates good performed gradually release properties and good prolonged pharmacological efficacy for 24 h which leads to high treatment efficacy, better availability of the drug and with expected improved extrapyramidal side effects.

Table 5
Pharmacokinetic parameters of risperidone following the administration of tested formulations Risperidal® tablet (4 mg) and floating microparticles F31.

Pharmacokinetic parameter	Tested formula	
	Risperidal® tablet (4 mg) (mean ± SD)	Floating microparticles (F31) (mean ± SD)
C_{max} (ng/mL)	254.695 ± 12.049	139.517 ± 9.097
[a]T_{max} (hr)	3	4
AUC_{0-24} (ng hr/mL)	1215.324 ± 171.964	1487.950 ± 285.178
$AUC_{0-\infty}$ (ng hr/mL)	1386.418 ± 189.014	1704.216 ± 384.623
$AUMC_{0-24}$ (ng hr/mL)	8822.582 ± 1605.656	12787.400 ± 3334.241
$AUMC_{0-\infty}$ (ng hr/mL)	15115.760 ± 3514.563	20478.270 ± 7477.428
MRT (hr)	10.851 ± 1.874	11.703 ± 2.118

SD = standard deviation, n = 6.
[a] Median value.

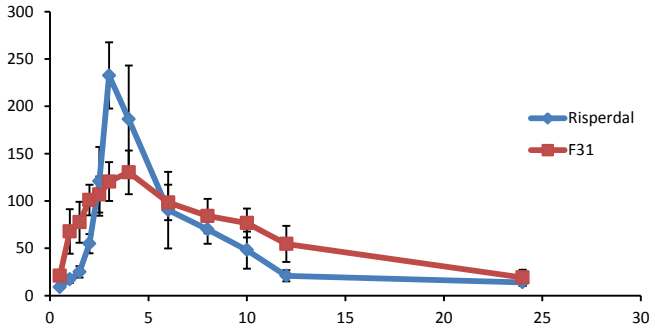

Fig. 15. Mean plasma concentrations of risperidone in six volunteers after administration of Risperidal® tablet (4 mg) and selected floating microparticles (F31).

3.5. Questionnaire

In the present study, a questionnaire was carried out by six human volunteers to study the extrapyramidal side effects of risperidone after administration of tested formulations Risperidal® tablet (4 mg) and floating microparticles (F31).

The questionnaire was developed to measure the symptoms associated with the side effects that they had expert after administration of an oral risperidone dosage form.

Table 6
Symptoms percent for the human volunteers after administration of risperidone market product and floating microparticles (F31).

	Score level	Symptoms percent for human volunteers taken:	
		Market product	Formula F31
Headache	0	0	0
	1	0	83.33
	2	0	16.67
	3	0	0
Drowsiness	0	0	33.33
	1	16.67	66.67
	2	50.00	0
	3	33.33	0
Nausea	0	0	66.67
	1	33.33	33.33
	2	50.00	0
	3	16.67	0
Tiredness	0	0	50.00
	1	0	33.33
	2	33.33	16.67
	3	66.67	0
Difficulty staying awake during the day	0	0	0
	1	0	100.00
	2	0	0
	3	100.00	0

Table 6 illustrates side effects scoring for the human volunteers after administration of the tested formulations.

By analysis of the results, we can evaluate the extrapyramidal side effect of risperidone after administration of risperidone market product and floating microparticles (F31) (Table 6). It was observed that the severity of symptoms after administration of floating microparticles (F31) showed marked decrease in the drug extrapyramidal side effect when compared to that of risperidone market product.

These data prove that floating microparticles have significant ($p < 0.05$) powerful effect to decrease the risperidone extrapyramidal side effect compared to that of market product and thus could dramatically improve the patients quality of life.

4. Conclusion

The investigated floating microparticles F31 containing 0.1 g risperidone, 0.8 g Eudragit S100, 0.2 g HPMC E50, 0.5 g Compritol 888 ATO and drug to MβCD in M ratio of 1:4 showed promising *in-vitro* and *in-vivo* properties and can be considered as a suitable candidate that could successfully achieve the targeted modified drug release and maintain plasma drug concentration required with excellent floating ability which persisted 95.93% for 12 h to make a significant improvement in patient's psychotic symptoms by its high therapeutic efficacy as well as overcoming the extrapyramidal side effect of risperidone.

Declaration of interest

The authors do not have declaration of interest.

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