CARBOPOL®-GUAR GUM GEL AS A VEHICLE FOR TOPICAL GEL FORMULATION OF PECTIN BEADS LOADED WITH RUTIN

PENSAK JANTRAWUT, WARINTORN RUKSIRIWANICH

Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.
Email: pensak.amuamu@gmail.com

ABSTRACT

Objective: The aim of this study was to develop suitable pharmaceutical gel formulations of pectin beads using semisynthetic celluloses or synthetic carbomers as a gelling agent.

Materials and Methods: Low methoxyl pectin (LMP) beads loaded with rutin composing 3% non-amidated LMP, 15% sorbitol and 1% sodium bicarbonate with 2% w/v of rutin were prepared, characterized, incorporated in various pharmaceutical gel bases and evaluated for rabbit skin irritation by close patch test.

Results: Rutin wetted bead formulation which showed spherical shape (around 700 μm), high rutin encapsulation efficiencies (82.02±0.91%), low conductivity (900 μS at 30 minutes) and faster rutin release (more than 80% in 30 minutes) was selected to incorporate in gel. The wetted rutin beads exhibited good stability (beads suspended in gel without breaking or gel color changing) in the gel composed of 0.4% w/v Carbopol® Ultrez 21 with 0.04% w/v guar gum at pH 5.0.

Conclusion: Rutin wetted beads in developed pharmaceutical gel formulation showed higher percentages of rutin remaining when stored at room temperature (27±2°C) than non-loaded rutin of about 1.53 times and gave no irritation in rabbit skin irritation test, however, the safety on human skin applications should be confirmed.

Keywords: Carbopol®, Guar gum, Pharmaceutical gel, Pectin, Rutin, Beads.

INTRODUCTION

Pharmaceutical gels are semi-solid preparations consisting of dissolved or dispersed active compound in either a hydrophilic or hydrophobic base with a gelling agent and other components. The number of newly formulated pharmaceutical gel products containing drugs and chemicals continues to increase every year [1-3]. The pharmaceutical gel formulation requires the use of an appropriate gelling agent with preferred characteristics include the inertness, safety, compatibility with other ingredients, good adhesion, permission of drug permeation while not being absorbed into blood circulation and irritation-free [4]. Many gelling agents have been commercially employed in the preparation of topical gels, including the semisynthetic cellulose derivatives [5,6] and synthetic carbomers [7]. Cellulose derivatives such as methyl cellulose (MC), hydroxypropylmethyl cellulose (HPMC), sodium carboxymethyl cellulose (SCMC) are the most popular cellulose derivative gelling agents used in pharmaceutical gel formulations. These semisynthetic cellulose derivatives are less sensitive for microbial contamination than natural gelling agents such as tragacanth, gum acacia, sodium algininate and gelatin. Carbopol® is a synthetic polymer gel consisting of polyacrylic acid crosslink subunit. Because of the synthetic nature of Carbopol®, the physical properties are highly controllable and reproducible during manufacturing and higher stability than natural gelling agents when prepare as gel [8].

Pectin, anionically charged structural plant polysaccharide consisting of a linear backbone of α-(1-4)-d-galacturonic acid residue [9], is an interesting candidate for pharmaceutical use as a carrier of a variety of drugs for controlled release applications. Pectin forms water-insoluble complexes with several drugs and be useful for sustained-release preparations [10-12]. Many techniques have been used to manufacture the pectin beads, especially ionotropic gelation. These simple techniques, together with the very safe toxicity profile, starting with the pectin droplets came in contact with the crosslinking solution (calcium chloride or zinc chloride solutions) after that the ionic interaction was occurred and formed gelled sphere beads. Rutin was selected as a model drug in this study. Rutin (quercetin-3-*O*-rutinoside), the flavonol glycoside of quercetin, is abundantly found and distributed in plants. It presents important properties in human health like its significant scavenging properties and anti-inflammatory activity [13,14] which will be beneficial for further develop as pharmaceutical gel formulation. The aim of this study was to develop suitable pharmaceutical gel formulation of pectin beads using semisynthetic celluloses or synthetic carbomers as a gelling agent. The physicochemical stability as well as rabbit skin irritation test of the selected pharmaceutical gel containing rutin loaded in pectin beads were evaluated.

MATERIALS AND METHODS

Materials

Non-amidated low methoxyl pectin (LMP) (Unipectine OF300C; DE=30% and DA=0%) were purchased from Cargill™ (Saint Germain, France). Rutin hydrate, sodium bicarbonate (NaHCO₃), sodium luaryl sulfate (SLS), Carbopol® ETD 2020 and Ultrez 21 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sorbitol pure anhydrate, Carbopol® 940 was purchased from Cooperation Pharmaceutique Francaise (Melun, France). HPMC, SCMC and guar gum were provided from Colorcon Limited (Orpington, UK). All reagents were analytical grade.

Preparation of LMP beads by encapsulator

Rutin loaded in the beads using the ionotropic gelation technique [15-17] was modified. The bead formulation which would obtain the soft texture was selected from our previous study [18]. Wetted beads were kept in 1% concentrated paraben solution at 4±2°C whereas dried beads were dried at 37±2°C for 24 hr in a drying room.

Wetted and dried LMP beads loaded with rutin characterization

Morphological studied

Morphological examinations of wetted and dried LMP beads were conducted by CETI® Microscope (CETI® Stereoscopic zoom microscope with built-in high resolution CCD-camera, Berkshire, United Kingdom). The experiments were performed at magnifications ×200. Size and shape of wetted and dried beads approximately 30 samples were evaluated by Xli Cap V18 Camera software (XL Imaging Limited, SA2 8PP, UK).

Water content determination

The water contents of wetted and dried beads were determined using moisture analyzer at 160°C until a stabilization of weight was achieved (OHAUS MB35, Greifensee, Switzerland).

Encapsulation efficiency

The amounts of rutin loaded into wetted and dried beads were determined by adding 100 mg of each bead into 1 L of phosphate buffer (PB) pH 7.4 for 3 hr until all of them were disintegrated. The solution was filtered and measured the absorbance at 267 nm by ultra violet (UV) spectroscopy (Biochrom Libra S22, Cambridge, UK). Rutin contents were determined from the standard curve of rutin in PB, which demonstrated linear with high correlation (r^2=0.9989). The following regression equation was obtained: y=0.0259 x+0.0108, where y was the absorbance and x was the concentration of rutin (mg/L). The experiment was done in triplicate. The percentages of encapsulation efficiencies were calculated according to the following equation: Encapsulation efficiency (%)=AQ/TQ×100. Where AQ is the actual quantity of rutin present in the matrices and TQ is the theoretical quantity of rutin.

Conductivity measurement

The relationship between conductivity and electrolytes which occurred when wetted and dried beads contacted the vehicle was investigated by conductivity meter (JENWAY, 4520, Essex, CM6 3LB, UK). Briefly, 100 mg of each bead was added into 50 ml of deionized water and measured the conductivity at 0, 0.5, 1, 1.5, 2 and 8 hr. The conductivity (µS) of wetted or dried beads in term of relative conductivity of the solution was calculated according to the following equation: The conductivity (µS) = (The conductivity of solution at several times – the conductivity of deionized water)/the weight of beads. The experiment was done in triplicate.

In vitro rutin release

The releases of rutin from wetted and dried bead were investigated using an *in vitro* rotating paddle dissolution apparatus (Sotax AT7, Binningerstrasse, Allschwil). The dissolution study was performed in PB (KH$_2$PO$_4$/1N sodium hydroxide [NaOH]) pH 7.4 at a rotation speed of 50 rpm and a temperature of 37±0.2°C. The accurately weight of beads (100 mg) were added to 1 L of dissolution medium. Samples were withdrawn at various time intervals up to 180 minutes by automatic pump and analyzed spectrophotometrically at 267 nm. All dissolution runs were performed in triplicate.

Gel preparation

Gels containing of HPMC, SCMC and guar gum at concentrations 1-5% w/v were prepared by dissolving each gelling agent in the deionized water with vigorous stirring using magnetic stirrer (stirrer, Heidolph SO 111, Germany). For MC, approximately half of the required volume of deionized water was heated to ~90°C before MC powder was stirred in and thoroughly wetted. The remainder of cold deionized water (~4°C) was then added to the heated-MC mixture and gently stirred. Following manufacture, all gels were placed in a vacuum to remove entrapped air. For Carbopol® 940, ETD 2020 and Ultrez 21, 0.1-0.5% w/v was added to deionized water and mixed using magnetic stirrer. The mixture was agitated for 30 minutes and the dispersion was then allowed to hydrate

and swell for 60 minutes. The pH of the unneutralized Carbopol® were measured to be 2.9-3.3. Then, the Carbopol® dispersion was neutralized with 98% triethanolamine (TEA) until the desired pH value was approximately reached (5.0, 6.0 and 7.0). During neutralization, the mixture was stirred gently with a spatula until homogeneous gel was formed. The selected Carbopol® were added guar gum (0.04% w/v of formulation) then stirred in water and followed the same preparation protocol that mention above. All gels were added 1% concentrated paraben as preservative and allowed to equilibrate for at least 1 hr at room temperature then stored at 4±2°C until use for further experiment. Finally, 0.01% wetted LMP beads loaded with rutin were incorporated into the prepared gel bases.

Physicochemical stability of the gel formulations containing LMP beads loaded with rutin

Physical stability

The gels containing rutin wetted beads were put in transparent tight containers and stored at room temperature (27±2°C) for 3 months. The physical characteristics including the bead characteristic, gel color, gel phase separation and sedimentation of beads were evaluated.

Chemical stability

The percentages remaining of rutin contents in wetted beads in the selected gel formulations at 0-3 months were determined by high-performance liquid chromatography (HPLC). The HPLC conditions of rutin acid were LC1200 ultra violet-visible detector and LC1100 HPLC pump (AS 1000, Thermo Finigan, USA) using Luna® C18, 10 µm i.d., 250×4.0 nm, Phenomenex USA column and a mobile phase containing H$_2$O/MeOH (1:1, v/v) at the flow rate of 1 ml/minutes. The 2-3 beads were collected from the gel, ground in PB pH 7.4 until disintegrated and filtered through a 0.45 µm membrane filter to obtain clear solution, prior to injection onto the HPLC column. An amount of 20 µl of the samples was injected into the column and monitored at 267 nm UV detector. The rutin contents were determined from the standard curve of the standard rutin, which demonstrated linear with high correlation (r^2=0.9994). The following regression equation was obtained: y=0.0228x+0.0698, where y was the peak area and x was the quantity of rutin (µg). The experiment was done in triplicate.

Viscosity measurements

A viscosimeter (Brookfield digital viscosimeter DV II RVTDV-II USA) was used to measure the viscosities (in pascal-second [Pa·s]) of the selected gel. The spindle (numbers of TF 96) was rotated at 10 rpm. Sample of the selected gel was settled over 30 minutes at the assay temperature (25±2°C) before the triplicate measurements were taken.

pH measurements

The pH was measured in each gel, using a pH meter (Philips PW 9422, UK), which was calibrated before each use with buffered solutions at pH 4, 7 and 10.

Rabbit skin irritation test by the closed patch test of the selected gel formulations containing LMP beads loaded with rutin

Three male rabbits (New Zealand White, 1.5-2.5 kg) were kept carefully following an acclimation period of 7 days to ensure their suitability for the study within a limited-access rodent facility with environmental conditions set to 25±2°C, 60-90% RH and 12 hr light/12 hr dark cycle. Animals were provided ad labium access to a commercial rabbit diet and the drinking water was supplied to each cage. Back of the animals was shaved to be free of fur with an electric clipper 24 hr before sample application. The shaved areas were divided into 10 sites of 2.5×2.5 cm each. An amount of 0.5 g of each sample and 5% SLS solution (positive control) was placed on each site. For gel containing rutin beads, the wetted beads were crushed by fingers before applied. The untreated site was used as a negative control. The treated sites were covered with gauze and the wrapped with a non-occlusive bandage. After 24 hr, the

bandage and the test samples were removed and the treated sites were washed 2 times with distilled water and air dried. One hour later, the sites were examined by optical visualization and measured by Mexameter® (Courage & Khazaka, Cologne, Germany) for skin edema and erythema. Scoring of erythema and edema was performed at 24, 48 and 72 hr [19] (and adopted by OECD Test Guideline 404). The primary irritation index (PII) was calculated using the following equation: PII = ([∑ erythema grade at 24/48/72 hr+∑ edema grade at 24/48/72 hr]/3×number of animals). The irritation degree was categorized based on the PII values as negligible (PII=0-0.4), or slight (PII=0.5-1.9), moderate (PII=2-4.9) or severe (PII=5-8) irritation. This study protocol has been reviewed and approved by the Ethical Committee of Faculty of Medicine, Chiang Mai University in Thailand.

Statistical analysis

All data were presented as mean ± standard deviation. The Kruskall–Wallis test was used to evaluate the significance of differences at the significant level of $p < 0.05$. Statistical analysis was performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Beads preparation and morphological studied

All beads were spherical shape with around 550 μm for dried and 700 μm for wetted bead size. The micrographs of the wetted and dried beads are presented in Fig. 1. The percentages of water contents were 89.90±2.13 and 13.78±0.89% in wetted and dried beads, respectively. The wetted beads exhibited higher percentage of water content than the dried one, due to pectin which is the insoluble fibers produces a mesh-like structure that traps compounds or other liquid, like a sponge absorbs water [20].

Rutin encapsulation efficiencies of wetted and dried beads were 82.02±0.91 and 80.45±1.78%, respectively. Poor aqueous solubility of rutin was responsible for its high encapsulation in the beads [18]. Several gelling agents have a limit electrolyte capacity. Adding of electrolytes in higher concentrations (above 3-4%) can precipitate the polymer and breakup the gel system [21]. Thus, the conductivity measurement of wetted and dried beads should be evaluated for their compatibility to the gels. Conductivity which measure of the concentration of ions in solution is proportional to the current that flows between the electrodes. Ions must be present in solution to carry the charge from one electrode to another. Increasing the number of ions in solution will increase the amount of charge that can be carried between electrodes and will increase the conductivity. The conductivities of rutin wetted and dried beads in deionized water are presented in Fig. 2. Both wetted and dried beads were reached their maximum conductivity about 900 and 1500 μS, respectively in 30 minutes. Dried beads exhibited higher conductivity than wetted one of about 1.67 times. The result indicated that the bead drying process was affecting significantly the amount of ions retained especially on the bead surface that also explained on higher conductivity found in the rutin dried beads. The drying process of beads may be resulted in the increase of salt concentration ($CaCl_2$) of the residual solution then the super-saturation increases and induced salt crystal growth.

The rutin releases of wetted and dried beads were assessed *in vitro* in phosphates dissolution media (pH 7.4). Fig. 3 demonstrate the release behavior of rutin from wetted and dried pectin beads in PB. Although, using PB as dissolution media may not be accessed the actual release profiles of pectin beads according to the ion exchange between Ca^{2+} and K^+ and disintegration of pectin matrix [22,23]. Many drug release profiles were anticipated by using PB [24,25]. Obviously, the release of rutin from wetted was faster than dried bead formulation. Initially, a rapid release was observed in wetted beads at 5 minutes while dried beads required at least 15 minutes of lag time. More than 60% of rutin in wetted beads was released at 30 minutes, compared with >40% released from dried beads at 1 hr. Rutin release from hydrophilic matrices such as pectin that used in the current study will be controlled

by the rate of hydration of bead matrix. In hydrophilic matrix systems, the carrier on the surface of the matrix initially hydrates during dissolution to generate an outer viscous gel layer. This phase is then sequentially followed by matrix bulk hydration, swelling and erosion. If the matrix does not hydrate, the outer viscous gel layer cannot be formed immediately, which may take more time of drug release during the initial phase. The hydration of polymer in a hydrophilic matrix system during dissolution has profound critically influence on drug release [26-28]. In conclusion, rutin loaded in wetted bead formulation which demonstrated low conductivity and faster rutin release behavior was selected to incorporate in various gel formulations then evaluate for the physicochemical stability and rabbit skin irritation.

Physicochemical stability of the selected gel formulations containing wetted LMP beads loaded with rutin

In recent decades, synthetic and semi-synthetic macromolecules are mostly used as gelling agents in pharmaceutical dosage forms. Some
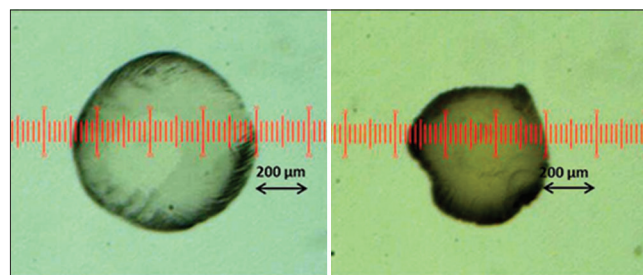

Fig. 1: The morphology of wetted (left) and dried (right) low methoxyl pectin beads loaded rutin observed under the microscope at magnifications ×200
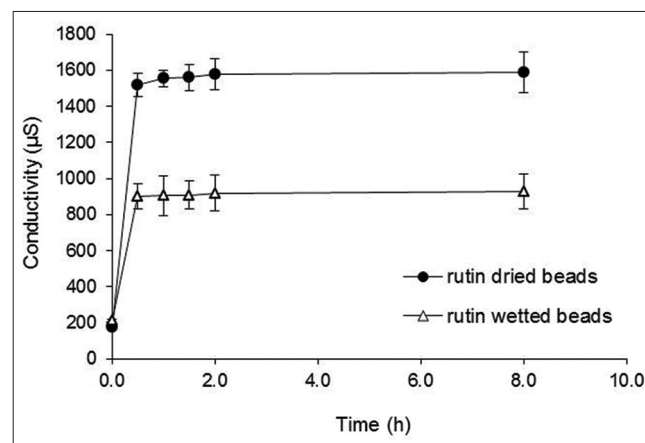

Fig. 2: Conductivities of rutin wetted and dried beads in deionized water at several times
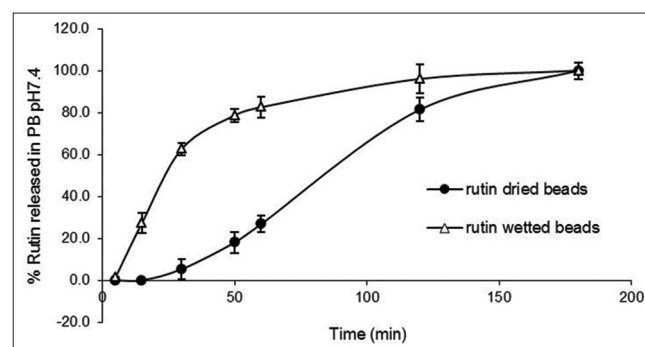

Fig. 3: Dissolution profiles of rutin wetted and dried beads in phosphates buffers

of these agents include: Carbomers, cellulose derivatives and natural gums. In this study, the gel bases including 4 and 5% w/v MC, HPMC and SCMC (pH 5.0), 2% w/v guar gum (pH 5.0), 0.4% w/v Carbopol® 940, ETD 2020 and Ultrez 21 (pH 5.0, 6.0 and 7.0), 0.4% w/v Carbopol® Ultrez 21 with 0.04% w/v guar gum (pH 5.0) gels were incorporated with 0.01% w/w wetted LMP beads loaded with rutin. Guar gum, a natural gelling agent, is mainly consisting of the high molecular weight polysaccharides and is obtained from the endosperm of the seed of the guar plant, *Cyamopsis tetragonaloba* (L) Taub. (syn. *Cyamopsis psoraloides*) [29]. 2% Guar gum gel base did not obtain clear gel (as compared to Carbopol® gel). Thus, the incorporated rutin beads were not seen in this gel. The characteristic of gels containing rutin wetted beads at room temperature after 1 month storage can be divided into four types (Fig. 4). The rutin beads were dropped down to the bottom of containers when using all cellulose derivatives as gelling agent (MC, HPMC and SCMC) at concentrations 4-5% w/v. Moreover, the cellulose derivatives gel bases were lost their viscosity and changed to be liquid (Fig. 4a). Minimum gel forming concentrations of cellulose derivatives are different based on the type and the molecular weights of them but the medium range is about 4-6% w/v. Adding higher concentrations (above 3-4%) of electrolytes can precipitate the polymer and breakup the gel system [21]. The electrolytes of the LMP beads including Ca^{2+} and Cl^- which remained from unwashed crosslinking solution may be responsible for breaking up the cellulose derivatives gel system. For synthetic Carbopol®, the rutin beads were dropped down and gel system were broken when using Carbopol® 940 at pH 5.0 and 6.0. Some rutin beads were broken when incorporated in 0.4% w/v Carbopol® ETD 2020 and Ultrez 21 gel bases at pH 5.0 (Fig. 4b) whereas Carbopol® gel bases at pH 6.0 and 7.0 showed the dissolving of rutin beads and the color of gels were changed to yellow which is the color of rutin (Fig. 4c). The best characteristic of gel and rutin beads was shown in 0.4% w/v Carbopol® Ultrez 21 with 0.04% w/v guar gum at pH 5.0 which rutin beads still suspended for 1 month at room temperature without bead breaking or color changing of the gel (Fig. 4d). Carbopol® polymers must be neutralized in order to achieve maximum viscosity. Unneutralized dispersions have an approximate pH range of 2.5-3.5 depending on the polymer concentration. Once a neutralizer such as NaOH or TEA is added to the dispersion, thickening gradually occurs. Optimum neutralization can be achieved in a pH range of 5.0-9.0 (Carbopol® data sheet, Novenon, TDS 237, Ohio, USA). The viscosity of Carbopol® gel is moderately sensitive to ions. Increased levels of monovalent ions will result in a decrease in viscosity, also di-or multi-valent ions (like Ca^{2+}) will precipitate Carbopol® polymers. Even if Carbopol® ETD 2020 and Ultrez 21 which provides superior electrolyte tolerance compared to

most Carbopol®, the breaking rutin beads were observed. Adjusting pH of Carbopol® gel more than 6.0 can dissolve rutin beads because the viscosity of LPM pectin bead decrease as pH of system increase [30,31] and rutin is freely soluble in alkaline conditions [32-34]. The rutin beads can be suspended when added low amount of guar gum into Carbopol® Ultrez 21. Thus, using guar gum as stabilizer imparted the important function of gel base which resulting in improved stability and reduced degradation of rutin beads in electrolyte containing system. Mechanism that is commonly exploited to improve stability included large size of stabilizer such as guar gum adsorb on the particle, creating bulky moieties on the particle surface. These moieties prevent particle-particle, particle-electrolyte interactions which results in limited particle aggregation and degradation [35,36].

The gel composing of 0.4% w/v Carbopol® Ultrez 21 with 0.04% w/v guar gum at pH 5.0 incorporated with rutin wetted beads was selected for further physicochemical stability evaluations. After 3 months storage at room temperature (27±2°C), the selected formulation gave good physical stability with no bead degradation and sedimentation, no gel layer separation and no gel color change. The viscosity of the selected gel incorporated with rutin wetted beads was measured and no significant different between the viscosity before (5.79±0.40 Pa·s) and after (5.84±0.17 Pa·s) storage. Fig. 5 showed the percentage remaining of rutin in the selected gel formulations at room temperature for 3 months. The percentages of rutin remaining in non- and loaded in LMP beads were 65.00±3.91 and 80.99±1.32%, respectively. Rutin beads incorporated in the selected gel formulation showed higher percentages of rutin remaining than that not loaded in LMP beads of about 1.53 times. In general, microencapsulation is one of the most frequently employed techniques used to overcome the stability problems of active components in order to protect them from oxidation providing an increased shelf life [37-39].

Rabbit skin irritation evaluation by the closed patch test

The calculated PIIs of all rutin bead gel formulations in rabbit skin irritation by the closed patch test at 24-72 hr were in the range of 0.22-0.11 except the rutin solution (PII=0.34-0.64, slight irritation) and the positive control (5% SLS, PII=1.22-0.78, slight irritant) (Table 1). The result for erythema of gels containing wetted beads was not significantly different from untreated area (negative control) after patch removal, although the increase of PIIs can be observed. Thus, these developed gel formulations gave no irritation. Since the gel base which was composed of 0.4% w/v Carbopol® Ultrez 21 together with 0.04% w/v guar gum gave no skin irritation. The irritation was from rutin which in the material safety data sheet, rutin may cause allergic reaction or irritation to the skin (Sunrise Science Products, CA, USA). Moreover, the higher PII of rutin solution may be also due to its pH 7.0 which more than the optimum pH balances of skin (pH 5.5) [40]. This has also supported this slight skin irritation results. However, the gels containing rutin wetted beads showed no irritation in rabbit skin, because of the reduction of the direct contact between rutin and
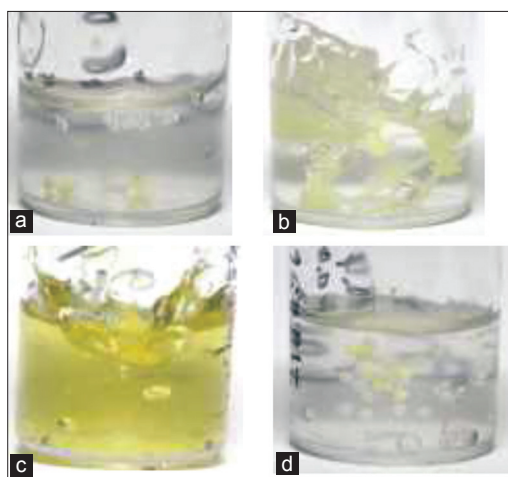

Fig. 4: Characteristic of gels containing rutin wetted beads at room temperature for 1 month: (a) Rutin beads were dropped down to the bottom, (b) rutin beads were broken, (c) rutin beads were dissolved into gel base (d) rutin beads were suspended in gel base
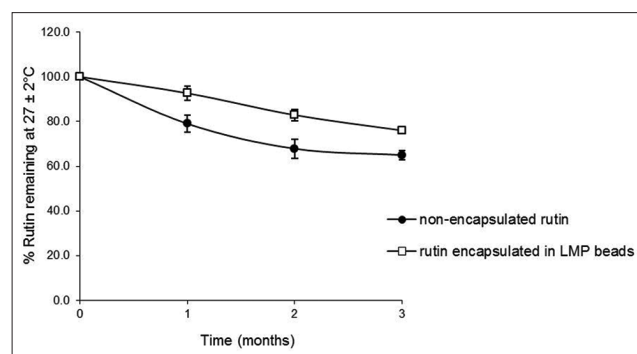

Fig. 5: The percentages of rutin remaining of non- and loaded in low methoxyl pectin beads and incorporated into the selected gel formulation at room temperatures (27±2°C) storage for 3 months

Table 1: PII and category of irritation based on PII of gel formulations containing wetted beads loaded with rutin and rutin solution

Samples	Primary irritation index (PII)			Category of irritation based on PII
	24 hr	**48 hr**	**72 hr**	
Gel base	0.11	0.11	0.00	Negligible
Gel containing rutin beads	0.22	0.11	0.11	Negligible
Rutin solution	0.34	0.58	0.64	Slight irritation
5% Sodium lauryl sulfate (positive control)	1.22	1.00	0.78	Slight irritation
Untreated area (negative control)	0.00	0.00	0.00	Negligible

Grading scale for skin irritation effect following OECD Test Guideline 404. PII=([$\sum$ erythema grade at 24/48/72 hr+$\sum$ edema grade at 24/48/72 hr] /3×number of animals). Gel base: 0.4% w/v Carbopol® Ultrez 21 together with 0.04% w/v guar gum gel base; Gel containing rutin beads: Gel base containing 0.01% wetted low methoxyl pectin beads loaded with 2% rutin (pH 5.0); rutin solution: 2% rutin in phosphate buffer solution (pH 7.0). PII: Primary irritation index

the skin. Thus, this gel formulation containing rutin beads could be regarded as safe for applications on skin. Furthermore, the *in vivo* skin irritation evaluation in human volunteers should be done in order to confirm the safety for human skin applications.

CONCLUSION

LMP beads loaded with rutin composing 3% non-amidated LMP, 15% Sorbitol and 1% $NaHCO_3$ with 2% w/v of rutin were prepared using the encapsulator. Wetted and dried beads loaded rutin were examined morphology, water content, encapsulation efficiency, conductivity and *in vitro* rutin release. Then, various gelling agents including 1-5% w/v of HPMC, SCMC, guar gum and 0.1-0.5% w/v of Carbopol® 940, ETD 2020 and Ultrez 21 were prepared and incorporated with rutin beads. The results found that rutin wetted beads demonstrated low conductivity, faster rutin release behavior and were selected to incorporate in various gel formulations. The selected gel which composed of 0.4% w/v Carbopol® Ultrez 21 with 0.04% w/v guar gum at pH 5.0 gave good stability (beads suspended in gel without breaking or gel color changing) and no irritation on rabbit skin irritation.

REFERENCES

1. Kikwai L, Babu RJ, Prado R, Kolot A, Armstrong CA, Ansel JC, *et al.* *In vitro* and *in vivo* evaluation of topical formulations of spantide II. AAPS PharmSciTech 2005;6(4):E565-72.
2. Dua K, Sara UV, Ramana MV, Agrawal A, Garg V, Soni N, *et al.* Formulation and evaluation of topical bases of aceclofenac. Indian Pharm 2006;321:117-23.
3. Choi JS, Shin SC. Preparation and evaluation of pranoprofen gel for percutaneous administration. Drug Dev Ind Pharm 2007;33(1):19-26.
4. Zatz JL, Kushla GP. Gels. In: Lieberman HA, Rieger MM, Banker GS, editors. Pharmaceutical Dosage Forms: Disperse Systems. 2nd ed., Vol. 2. New York: Marcel Dekker; 1996. p. 399-421.
5. Mohammed FA. Topical permeation characteristics of diclofenac sodium from NaCMC gels in comparison with conventional gel formulations. Drug Dev Ind Pharm 2001;27(10):1083-97.
6. Tas C, Ozkan Y, Savaser A, Baykara T. *In vitro* and ex vivo permeation studies of chlorpheniramine maleate gels prepared by carbomer derivatives. Drug Dev Ind Pharm 2004;30(6):637-47.
7. Patel M, Patel B, Patel R, Patel J, Bharadia P. Carbopol; a versatile polymer. Drug Deliv Technol 2006;6:32-4.
8. Wade A, Weller P. Handbook of Pharmaceutical Excipients. 2nd ed. London: American Pharmaceutical Association, The Pharmaceutical Press; 1994. p. 71-3.
9. Voragen AG, Pilnik W, Thibault JF, Axelos MA, Renard CM. Pectins. New York: Marcel Dekker Inc.; 1995.
10. Takahashi Y, Nambu N, Nagai T. Interaction of several nonsteroidal anti-inflammatory drugs with pectin in aqueous solution and in solid state. Chem Pharm Bull 1978;26(12):3836-42.
11. McMullen JN, Newton DW, Becker CH. Pectin-gelatin complex coacervates II: Effect of microencapsulated sulfamerazine on size, morphology, recovery, and extraction of water-dispersible microglobules. J Pharm Sci 1984;73(12):1799-803.
12. Naggar VF, E1-Khawas M, Ismail FA, Boraie NA. Pectin, a possible matrix for oral sustained-release preparations of water soluble drugs. STP Pharm Sci 1992;3:227-34.
13. Verma AR, Vijayakumar M, Mathela CS, Rao CV. *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. Food Chem Toxicol 2009;47(9):2196-201.
14. Itagaki S, Oikawa S, Ogura J, Kobayashi M, Hirano T, Iseki K. Protective effect of quercetin-3-rhamnoglucoside (rutin) on ischemia-reperfusion injury in rat small intestine. Food Chem 2010;118(2):426-9.
15. Aydin Z, Akbuga J. Preparation and evaluation of pectin beads. Int J Pharm 1996;137(1):133-6.
16. Bourgeois S, Gernet M, Andremont A, Fattal E. Design and characterisation of pectin beads for the colon delivery. Proceedings 4th World Meeting ADRITELF/APGI/APV, Florence; 2002. p. 805-6.
17. El-Gibaly I. Oral delayed-release system based on Zn-pectinate gel (ZPG) microparticles as an alternative carrier to calcium pectinate beads for colonic drug delivery. Int J Pharm 2002;232(1-2):199-211.
18. Jantrawut P, Assifaoui A, Chambin O. Influence of low methoxyl pectin gel textures and *in vitro* release of rutin from calcium pectinate beads. Carbohydr Polym 2013;97(2):335-42.
19. Draize JH, Woodard G, Calvery H. Methods for the study of irritation and toxicity of substances applied to the skin and mucous membranes. J Pharmacol 1944;82(3):377-90.
20. Corley JR, Easter RA, Roos MA, Fahey GC Jr. Effect of various fiber sources on gain feed efficiency and nitrogen retention in the weanling pig. Nutr Rep Int 1978;18(2):135-42.
21. Allen LV, Popovich NG, Ansel HC. Ansels Pharmaceutical Dosage Forms and Drug Delivery Ayatems. 8th ed. United States of America: Lippincott Williams & Wilkins; 1995. p. 63-7.
22. Sriamornsak P. Investigation of pectin as a carrier for oral delivery of proteins using calcium pectinate gel beads. Int J Pharm 1998;169(2):213-20.
23. Das S, Ng KY, Ho PC. Formulation and optimization of zinc-pectinate beads for the controlled delivery of resveratrol. AAPS PharmSciTech 2010;11(2):729-42.
24. Ranjha NM, Mudassir J, Sheikh Z. Synthesis and characterization of pH-sensitive pectin/acrylic acid hydrogels for verapamil release study. Iran Polym J 2011;20:147-59.
25. Dutta RK, Sahu S. Development of oxaliplatin encapsulated in magnetic nanocarriers of pectin as a potential targeted drug delivery for cancer therapy. Results Pharm Sci 2012;2:38-45.
26. Wan LS, Heng PW, Wong LF. Relationship between swelling and drug release in a hydrophilic matrix drug. Dev Ind Pharm 1993;19(10):1201-10.
27. Mitchell K, Ford JL, Armstrong DJ, Elliot PN, Hogan JE, Rostron C. The influence of drugs on the properties of gels and swelling characteristics of matrices containing methylcellulose or hydroxypropylmethylcellulose. Int J Pharm 1993;100(1-3):165-73.
28. Colombo P, Bettini R, Santi P, Ascentiis A, Peppas NA. Analysis of the swelling and release mechanisms from drug delivery systems with emphasis on drug solubility and water transport. J Control Release 1996;39(2-3):231-2.
29. Belitz HD, Groseh W. Food Chemistry. Berlin: Springer-Verlag; 1987. p. 242-3.
30. Christian JB, Bryant SE. Ester content and jelly pH influences on the grade of pectin. J Food Sci 1968, 33(3):262-4.
31. Awasthi R. Selection of pectin as pharmaceutical excipient on the basic of rheological behavior. Int J Pharm Pharm Sci 2011;3(1):22931.
32. Miyake K, Arima H, Hirayama F, Yamamoto M, Horikawa T, Sumiyoshi H, *et al.* Improvement of solubility and oral bioavailability of rutin by complexation with 2-hydroxypropyl-beta-cyclodextrin. Pharm Dev Technol 2000;5(3):399-407.
33. Tommasini S, Raneri D, Ficarra R, Calabrò ML, Stancanelli R, Ficarra P. Improvement in solubility and dissolution rate of flavonoids by complexation with beta-cyclodextrin. J Pharm Biomed Anal 2004;35(2):379-87.

34. Chebil L, Humeau C, Anthoni J, Dehez F, Engasser JM, Ghoul M. Solubility of flavonoids in organic solvents. J Chem Eng Data 2007;52(5):1552-6.

35. Cosgrove T. Colloid Science-Principles, Methods, and Applications. Oxford: Blackwell Publishing; 2005.

36. Tiraferri A, Chen KL, Sethi R, Elimelech M. Reduced aggregation and sedimentation of zero-valent iron nanoparticles in the presence of guar gum. J Colloid Interface Sci 2008;324(1-2):71-9.

37. Kuhn GO, McCampbell P, Singmaster G, Arneson DW, Jameson CW. Application of microencapsulation technology to improve the stability of citral in rodent diets. Fundam Appl Toxicol 1991;17(3):635-40.

38. Favaro-Trindade CS, Pinho SC, Rocha GA. Revisão: Microencapsulação de ingredientes alimentícios. Braz J Food Technol 2008;11:103-12.

39. Sun-Waterhouse D, Zhou J, Miskelly GM, Wibisono R, Wadhwa SS. Stability of encapsulated olive oil in the presence of caffeic acid. Food Chem 2011;126(3):1049-56.

40. Schmid MH, Korting HC. The concept of the acid mantle of the skin: Its relevance for the choice of skin cleansers. Dermatology 1995;191(4):276-80.