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In Vitro and *In Vivo* Evaluation of Combined Time and pH- Dependent Oral Colonic Targeted Prednisolone Microspheres

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Authors' contributions

This work was carried out in collaboration between all authors. Author FMH designed the study and wrote the protocol. Author DSS wrote the first draft of the manuscript. Authors DSS and MN managed the analyses of the study, managed the literature searches. Author RR run experiments and performed the statistical analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: to enhance the anti-inflammatory effect as well as oral absorption of prednisolone (PR), through formulation of colonic targeted microspheres prepared from a blend of time and pH- dependent polymers and loaded with PR.
Study Design: *In Vitro* and *In Vivo* Evaluation of Combined Time and pH- Dependent Oral Colonic Targeted Prednisolone Microspheres.
Place and Duration of Study: Department of Pharmaceutics and Industrial Pharmacy, Helwan University, Cairo, Egypt between June 2011 and October 2012.
Methodology: Microspheres were prepared by solvent evaporation method using different ethyl cellulose (EC) and Eudragit® S-100 (ES100) ratios with 0.5 and 1% w/v span® 80 as emulsifier. The microspheres were evaluated for surface morphology, particle size, drug encapsulation efficiency % and *in vitro* drug release at pH 1.2 and 7.4. The anti-inflammatory activity of selected formula was compared to that of conventional PR tablets.
Results: A decrease in drug entrapment efficiency % was obtained with increasing both polymers and surfactant concentrations. Based on drug release results, the formula of 1:

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1: 0.16 w/w/w, EC: ES100: PR ratio with 1% w/v span® 80 was selected for further histopathological evaluation of the anti-inflammatory activity in colitis induced-rats. Histopathological study showed undefined tissue necrosis after treatment with the selected microspheres; however, diffused necrosis was observed in rats treated with the commercial tablets. *In vivo* absorption study showed that values of C_{max} and AUC_{0-24} of both formulations were insignificantly different. However, the occurrence of C_{max} of microspheres was significantly delayed in comparison to free drug (9.17 to 2.67hr) (*P*<.001).

Conclusion: This study has supplied us with brightening results concerning the therapeutic efficacy of a blend of time and pH- dependent polymers colonic targeted microspheres.

Keywords: Colonic microspheres; ethyl cellulose; eudragit S100; histopathological study; in vivo drug absorption.

1. INTRODUCTION

Various approaches have been reported to develop site-specific drug delivery to the colon [1,2]. Methods based on pH- sensitive delivery system such as enteric coated dosage forms could be simple and practical. The commonly used pH- responsive polymer to target drugs to the colonic region was the methacrylic acid and methyl methacrylate ester copolymer marketed as Eudragit S [3,4,5].

Several drugs were coated with pH dependent polymers; Eudragit S and Eudragit L, for targeting the colonic region [6,7]. Although Eudragit S is capable of protecting the drug during its transit through the upper gastrointestinal tract, it affords poor insufficient site specificity because most of the drug is released in the upper small intestine after gastric emptying even though drug release is effectively prevented in the stomach [8].

This lack of specificity led to evaluation of another method that depends on relative consistency of small intestinal transit times such as sustained release dosage forms [9]. These systems can be designed to deliver drugs to the colon. Ethyl cellulose is a non-biodegradable, hydrophobic polymer and extensively studied encapsulating materials for sustained release systems [10,11,12,13].

Time- dependent systems may overcome some of the difficulties associated with variable gastric pH, but inter-subject variability in gastrointestinal transit time, may give rise to difficulty for colonic drug delivery [14] in this approach the colon arrival time of dosage forms can't be accurately predicted, resulting in poor colonic availability.

Based on the physiological characteristics of the human gastrointestinal tract and the movement of dosage forms therein, it was reported that a colonic delivery system which is based only on time in the GI tract or pH- dependence wouldn't be acceptable because of the inherent variability of pH and emptying times for the GI tract. The advantage of relatively constant transit time of the small intestine (3- 4 hr) and the high pH of the distal small intestine (7- 8) could be used by certain authors for formulation of a reliable multi- unit colonic delivery system [15].

The objective of this study was to enhance the anti-inflammatory effect and oral absorption of prednisolone by formulation of colonic targeted microspheres containing prednisolone using a blend of time and pH- dependent polymers.

2. MATERIALS AND METHODS

2.1 Materials

Prednisolone (PR) at > 98.9 % purity was donated from Al Arabia pharmaceutical Company, Cairo, Egypt. Ethyl cellulose (EC) and carboy methylcellulose (CMC) were obtained from Sigma-Aldrich, Inc., Germany. Eudragit S 100 (ES100) was obtained from Heinrich's Commercial Agency of Evonik Rohm Pharma Polymers. Sorbitan monoleate (span® 80) was obtained from Fluka Biochemika Company, Sigma Germany. Methyl alcohol, 2, 4, 6-trinitrobenzenesulfonic acid (TNBS) was obtained from Sigma- Aldrich Co. Hostacortin H® Tablets contain 5mg prednisolone obtained from a local pharmacy store (Batch no. 10E44, Sanofi Aventis Pharmaceutical Co., Egypt). Chloroform, n-Hexane, Acetone, Sodium dihydrogen phosphate, disodium hydrogen phosphate, liquid paraffin, from The United Company for Chemicals and Medical Preparation, Cairo, Egypt. All other materials used were of pharmacopeial grade.

2.2 Preparation of Microspheres

The microspheres were prepared by solvent evaporation method [16]. Briefly, drug-polymers solution was prepared by dissolving different ratios of PR, EC and ES100 in 20 ml methanol as illustrated in Table 1. The drug- polymers mixtures were dropped into 100 ml light liquid paraffin containing span® 80 as an emulsifier in two different concentrations (0.5 and 1% w/v). The mixture was kept stirring at 4000 rpm for 5 hr. The obtained microspheres were then separated by centrifugation at 6000 rpm for 30 min (Hettich zentrifugen- EBA20-Germany) and washed successively with n- hexane to remove the adhering oil. The microspheres were allowed to dry at room temperature. Various formulation variables including the different polymers ratios as well as span® 80 concentrations that could affect the properties of the microspheres were identified.

2.3 Differential Scanning Calorimetry (DSC)

DSC was carried out on pure substances and on microspheres using the Shimadzu DSC-50 instrument (Shimadzu, Kyoto, Japan) to detect any interaction between prednisolone and the tested polymers. Samples (4-5 mg) were placed in an aluminum pan and heated at a rate of 10ºC/min with indium in the reference pan in an atmosphere of nitrogen to a temperature of 300ºC.

2.4 Particle Size Analysis

The mean diameter of the microspheres was determined using microvision image analysis system (APSI stage micrometer scale, England) using glycerin as the dispersion phase.

2.5 Microspheres' Surface Morphology

Surface morphology of the microspheres was evaluated by means of scanning electron microscope (JEOL, JXA 840a electron probe micro-analyzer, Japan).The microspheres were dried and coated with gold palladium and examined microscopically.

2.6 Encapsulation Efficiency %

Fifty milligrams of the drug loaded microspheres were suspended in 20 ml methanol. The suspension was shaken vigorously and kept overnight to allow the drug to be extracted into methanol. One milliliter of the suspension was filtered through 0.45 um membrane filter, suitably diluted and analyzed for the prednisolone content spectrophotometrically at 245 nm. The encapsulation efficiency (EE %) of the microspheres was calculated according to the following equation:

$$EE\ (\%) = [\text{Actual drug entrapped } * / \text{Theoretical drug content}] \times 100 \quad (1)$$

***** Actual drug entrapped = [Theoretical drug content - free drug]

2.7 *In vitro* Drug Release

In vitro drug release was conducted in a USP Dissolution Tester Apparatus II (Hanson Research, Chatsworth, USA) at a stirring speed of 100 rpm and temperature of 37°C. An amount of the prepared microspheres equivalent to 5 mg PR were added to the release medium. Initial drug release was conducted in 700ml 0.1N HCl for 2 hr, then 200ml of 0.2M tribasic sodium phosphate was added to the dissolution vessels and pH was adjusted to 7.4 using NaOH. Samples were withdrawn at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hr and replaced with fresh media. The withdrawn samples were filtered through 0.45 µm membrane filter and analyzed for PR content using UV spectrophotometer at 245nm.

2.8 *In vitro* Release Kinetics

In order to determine drug release mechanism from the prepared microspheres, the release kinetic data were analyzed according to Korsmeyer-Peppas release model [17] given by the following equation:

$$M_t/M_\infty = Kt^n \quad (2)$$

Where M_t is the amount of drug released at time t; M_∞ is the amount of drug released at infinite time; K is the kinetic constant related to the structural and geometric characteristics of the drug delivery system (microspheres); and n is the release exponent indicative of the release mechanism. The n values used for elucidation of drug release mechanism from the microspheres were determined from log cumulative percentage of drug release versus log time plots. Values of n near 0.5 indicate predominantly diffusion control and of 1.0 correspond to zero-order release. Another analysis mechanism was used considering that drug release in swellable matrices depends on two processes, drug diffusion into the swollen polymer and matrix swelling due to approximate contribution of the diffusion and relaxation mechanisms. This was carried out by fitting the data to the model proposed by Peppas and Sahlin [18] given by the following equation:

$$Mt/M\infty = K1tm + K2t2m \quad (3)$$

Where K_1 and K_2 are obtained from non linear regression curve fitting of the release data using Graph Pad prism4 (Graph Pad Software, San Diego, CA, USA). When $K_1 > K_2$, the release is mainly controlled by diffusion, and when $K_2 > K_1$, the release is mostly due to matrix swelling. When K_1 is nearly equal to K_2, the release is a combination of diffusion and polymer relaxation [19].

2.9 *In vivo* Study

The *in vivo* study protocol was reviewed and approved by the ethical committee of Faculty of Pharmacy, Helwan University. The study was conducted in accordance to EC Directive 86/609/EEC for animal experiments.

2.10 Histopathological Study

The histopathological study was carried out to evaluate the anti-inflammatory effect of the selected microspheres on rats subjected to experimental colitis. Sixteen male Wistar rats each weighing 200 g (6-8 weeks) were used throughout the experiment. The animals were divided into 4 groups (4 each); group I, normal control group; group II, induced colitis group, received no treatment; group III, rats treated with the selected prednisolone microspheres and group IV treated with conventional PR tablets (Hostacortin H®).

2.10.1 Induction of colitis

Rats were fasted for 24 hr with free access to water before the experiment. Colitis was induced in all rats except the control group following the method described in literature [20]. Briefly, a rubber catheter was inserted rectally into the colon. Colitis was induced by slow intra rectal administration of 2ml containing 135mg/kg TNBS dissolved in 50% ethanol. The rats were housed for 3 days without treatment to maintain the development of a full inflammatory bowel disease model.

2.10.2 Treatment and sampling

Rats of groups III and IV received oral PR microspheres (Selected formula) suspension and commercial PR tablets dispersed in 1% CMC solution, respectively in a dose equivalent to 5mg/kg once daily for five continuous days via gastric intubation. Rats of group II received the same volume of 1% CMC solution without drug. Autopsy samples were taken from the colon of rats in different groups and maintained in 10% (v/v) formalin in saline. Specimens were stained by hematoxylin and eosin for histopathological examination throughout the light microscope.

2.11 *In vivo* Absorption Study

Two groups of rats were fasted over night for 12 hours prior to the experiment with free access to water. Each group (6 rats) received a single oral dose of prednisolone (5mg/kg) by gastric intubation, where group I received free PR dispersion, while group II received the selected microspheres formula, both treatment doses were dispersed in 1% CMC solution.
Serial blood samples (1 ml) were collected directly through retro- orbital puncture from each rat into previously heparinized eppendorff tubes at 1, 3, 5, 7, 9, 10, 12 and 24 hr post dose.

Collected blood samples were centrifuged to separate plasma for analysis. Plasma levels of PR were analyzed using an HPLC method [21] and was adopted with some modifications. The HPLC system was Shimadzu 10A VP (Shimadzu degasser DGU 12A, Japan), pump (Model LC- 10A DVP, Shimadzu, Japan), an ultra- violet wavelength detector (Model SPD-10A VP, Shimadzu, Japan), a column (Teknokrama, C18, 5um, 25x 0.46) and system controller (Model SCL 10 VP). The mobile phase was made of Acetonitrile: water (70: 30% v/v). Analysis was run at a flow rate of 1.5 ml/ min. and the detection wavelength was at 245nm.

The pharmacokinetic parameters of the selected microspheres formula as well as the free PR were compared using plasma concentration (C_{max}), time to reach peak plasma concentrations (T_{max}) obtained directly from the plasma data, area under the plasma concentration- time curve (AUC_{0-24}) estimated by linear trapezoidal approximation and absorption rate constant (k_a) calculated using Wagner- Nelson method [22].

2.12 Statistical Analysis

The values of all the studied parameters of the selected microspheres formula were compared to the same parameters obtained from the free prednisolone using student- t test. A statistically significant difference was considered at *P* value <.05.

3. RESULTS AND DISCUSSION

3.1 Differential Scanning Calorimetry (DSC)

The DSC thermograms of PR, EC, ES100 and the prepared microspheres were presented in Fig. 1 (A, B, C and D, respectively). It is clear that the DSC thermogram of PR powder exhibits a single sharp characteristic, endothermic melting peak at 240.94ºC indicating its crystalline state. The DSC thermograms of EC, ES100 show endothermic broaden peaks at 284.33 and 208.77ºC, respectively. The DSC thermogram of the prepared microspheres shows that the characteristic melting peak of prednisolone had disappeared. This could be attributed to either the possible change of the drug to an amorphous form or due to molecular dispersion of the drug in the polymeric matrix. Similar results have been observed by many authors [23,24].

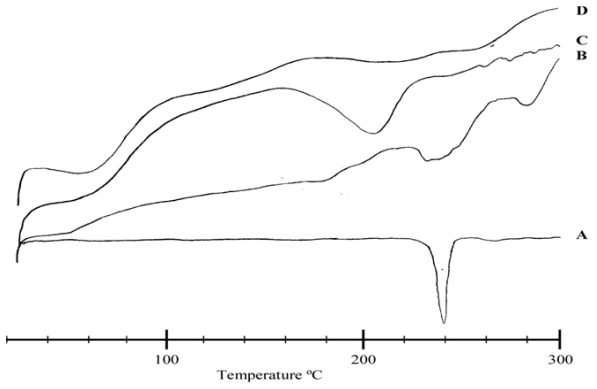

Fig. 1. DSC thermograms of (A) Prednisolone, (B) EC, (C) ES100 and (D) microspheres

3.2 Particle Size Analysis

The composition and mean particle size of the prepared microspheres were presented in Table 1. The results clearly revealed that the polymer concentration has significant impact on the average diameter of the prepared microspheres. The data was correlated to what has been previously reported in literature [25] where increasing the medium viscosity through increasing polymer concentrations resulted in the formation of larger size droplets and consequently larger sized-microspheres.

On the other hand, increasing span® 80 concentration from 0.5 to 1% resulted in decreasing microspheres diameter as presented in Table 1. The particle size means of the formulae prepared with 0.5 % w/v span® 80 (F5, F6 and F7) are significantly larger ($P < 0.05$) than those derived from their corresponding formulae prepared with 1% w/v span® 80 (F2, F3 and F4, respectively). This result was attributed to the inability of low concentration of surfactant to cover the entire organic droplet surface. Thereby, some of the droplets would tend to aggregate till the surface area of the droplet was decreased to such a point that the available amount of surfactant was able to coat the entire surface of the agglomerated and thus forming a stable emulsion with relatively larger droplets. Consequently, larger microspheres would be produced following solvent evaporation [26].

Table 1. Composition and characteristics of the prepared microspheres

Formula	EC:ES100:drug (w/w/w)	Span®80 (% w/v)	Mean particle size (µm)	EE %
F1	1:1:0.16	1%	135 ± 1.25	56 % ± 2.1
F2	2:1:0.16	1%	180 ± 1.8	52 % ± 1.6
F3	3:1:0.16	1%	225 ± 0.93	43 % ± 3.6
F4	4:1:0.16	1%	243 ± 0.77	35 % ± 1.8
F5	2:1:0.16	0.5%	252 ± 0.87	75 % ± 2.4
F6	3:1:0.16	0.5%	297 ± 0.56	55 % ± 2.8
F7	4:1:0.16	0.5 %	381 ± 1.34	47 % ± 3.2
F8	1:2:0.16	1%	196 ± 1.2	50 % ± 1.9
F9	0:3:0.16	1%	120 ± 0.82	57 % ± 3.7

EC: ethyl cellulose; ES 100: Eudragit S 100; EE: Encapsulation Efficiency; Values are expressed as mean ± SEM. n = 6

3.3 Microspheres' Surface Morphology and Encapsulation Efficiency

SEM photographs of PR loaded microspheres revealed that the surface structure of all prepared microspheres was characterized by regular spherical shape, having some pores on the surface (Fig. 2). No difference in the morphology of the resultant microspheres was observed upon changing concentrations of polymer and emulsifier (data not shown).

Table 1 showed the EE % of all the prepared microspheres formulae calculated by Eq. 1. The data indicated that, increasing EC concentration in the microspheres (F2, F3, F4, F6 and F7) resulted in a decrease in EE% irrespective to surfactant concentration. The possible explanation for the obtained results is the effect of increasing viscosity of the polymer solution at higher concentration leading to larger polymer/ solvent droplets, making it difficult

for the drug to diffuse inside the particles during preparation process and consequently decreasing the EE%. On parallel line, increasing ES100 concentration in microspheres insignificantly influence the EE % (F8 and F9). This could be revealed to the lower viscosity of ES100 in comparison to the viscosity of EC at the same concentration.

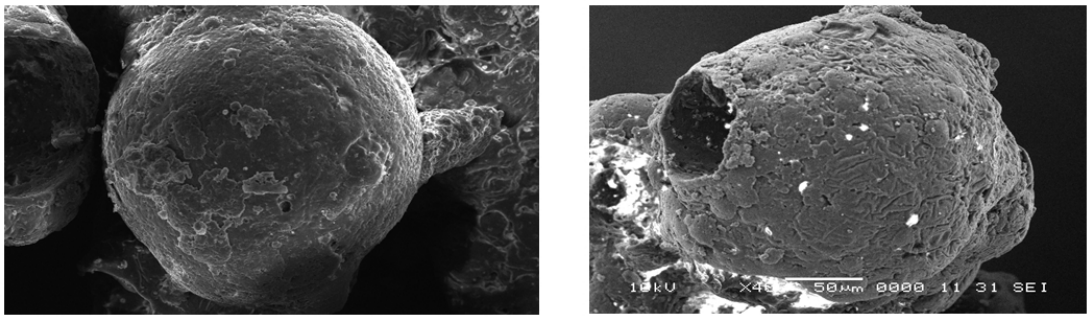

Fig. 2. Representative scanning electron microscopy photographs of prednisolone-loaded microspheres.

The microspheres (F5, F6 and F7) prepared using 0.5 % w/v span® 80 exhibited an overall increase in the EE% in comparison to microspheres (F2, F3 and F4) that prepared using 1 % w/v span® 80. This may be due to the higher surfactant concentration resulted in reduction in the particle size of the emulsion droplets and the formation of more micelles containing the drug. These micelles were more miscible with liquid paraffin oil, causing increased drug diffusion into the continuous phase [27].

3.4 *In vitro* Drug Release

Upon observing the release profiles shown in Fig. 3, it can be seen that a decrease in % drug release after 24 hr (2 h in 0.1N HCL pH 1.2, then in phosphate buffer pH 7.4 till the end of 24 hr) from formulae prepared with increasing EC to ES100 ratio and 1 % span® 80, F1-F4 (Fig. 3A). F1 (EC: ES100, 1:1 w/w) exhibited a nearly complete release (88%) within 24 hr of incubation in dissolution medium compared to only 65% with 4 folds increase in EC :ES 100 ratio (formula F4). The concentration of EC in the dispersed phase exerted a significant impact on drug release through increasing the microspheres particle size and consequently producing smaller surface exposed to the release medium, thus decreasing the % drug released. Another reason might be the increase in concentration of the hydrophobic polymer (EC) around the drug particles decreasing the wetability and hence the dissolution rate of the drug [28].

As the concentration of span® 80 decreased from 1 to 0.5% (w/v) (Fig. 3B), the amount of PR released from microspheres decreased. F2 (EC: ES100, 2:1 w/w and 1% w/v span® 80) exhibited a release of 84.5% (Fig. 3A) within 24 hr of incubation in dissolution medium compared to only 59 % drug release exhibited by F5 (EC: ES100, 2:1 w/w and 0.5% w/v span® 80) as presented in Fig. 3B. Similarly, the % drug released dropped from 71.5% and 59% with F3 and F4 (Fig. 3A) to 56% and 53% with F6 and F7, respectively with decreasing span® 80 concentration (Fig. 3B). As expected and from literature [11,29], the higher release rate of the drug could be attributed to the higher surfactant concentration that leads to a smaller particle size and consequently larger surface area available for the release medium.

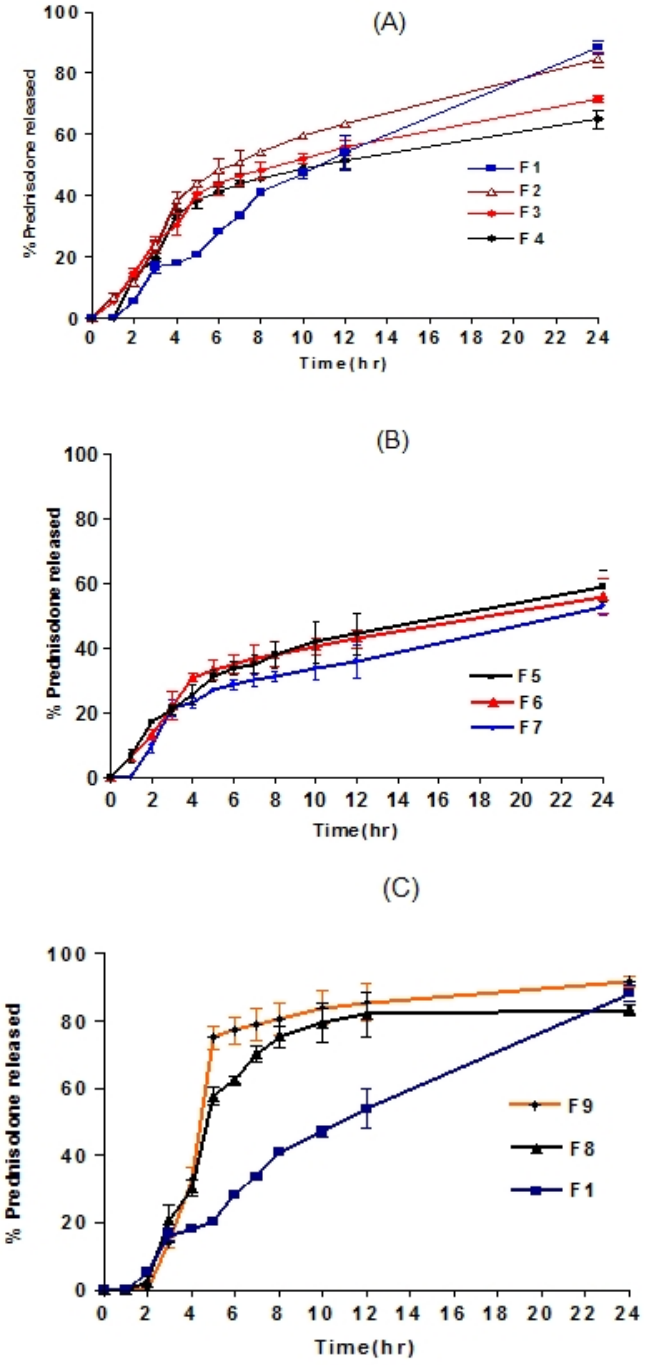

Fig. 3. *In vitro* release profiles of prednisolone from different microspheres prepared using different EC: ES100 ratios in presence of 1% span® 80 (A) or 0.5 % span® 80 (B) and microspheres prepared using increasing ES100 ratio and 1% span® 80 (C)

In contrast, increasing ES100 resulted in increasing % PR released in the first 5 hr of release (Fig. 3C). The release profiles showed that the formulae prepared by doubling ES-100 concentration from 1:1 w/w (F1) to 1:2 w/w (F8), using 1% w/v span® 80 had nearly similar % drug released after 24 hr (88.3 % and 83.3 %), respectively. On the other hand, regarding the first 5 hr (pH 1.2 for 2 hours then changed to pH 7.4), the % PR released was 20.6% and 57.6% from F1 and F8, respectively as seen in Fig. 3C. This result can be explained by the fact that increasing ES100 content produced pores that allow the diffusion of the dissolution medium to the microspheres which results in faster drug release in F8 (57.6%) when compared with lower ES100 content in F1 (20.6%) after 5 hours at pH 7.4. Formula F8 that released 57.6% of the drug after the 1st 5 hr (before reaching the colon) can be utilized for ileo-colonic drug targeting, which requires high ratio of ES100 as a pH dependent polymer, that is insoluble in the low pH environment (1- 2.5) and dissolves at the higher pH (7.5) of the distal gastrointestinal tract (in the distal small intestine) [30]. However, Formula F1 that only released 20.6% of the drug after the 1st 5 hr can be more promising for specific targeting of PR to the colon.

The formula prepared using ES100 only (F9) released 75 % and 91.8 % after 5 and 24 hr, respectively (Fig. 3C). This is a further proof that using ES100 only gives higher PR release after 5 hr (at pH 7.4), which necessitates the incorporation of EC as a time dependent polymer to control the release during the first 5 hours while keeping high % release after 24 hours.

The kinetics of PR release from the prepared microspheres was studied by fitting the release data up to 60% of PR to Korsmeyer- Peppas model (Table 2). It could be seen that the values of n for all formulae, except F1 and F8, were < 0.45 which indicates Fickian (case I) release. However; formulae prepared with higher ES100 concentration had n values > 0.89, indicating super case II transport. Further analysis by Peppas and Sahlin model showed that all the microspheres formulae, except formulae F1 and F8, had $K_1 > K_2$ indicating greater diffusional contribution, while F1 and F8 had $K_2 > K_1$ reflecting greater relaxational contribution and release is mostly due to matrix swelling. F1 and F8 showed super case II transport as well as relaxational contribution, which supports erosion rather than diffusion mechanism of drug release. This could be explained by the higher ratio of ES100 which releases the drug by erosion and not by swelling or diffusion.

Table 2. Fitting of release kinetic models to prednisolone release data

Formulations	Korsmeyer model*		Peppas-Sahlin model		
	n	r^2	K_1 (% $h^{-0.45}$)	K_2 (% $h^{-0.9}$)	r^2
F1	1.075	0.9605	-2.838	6.891	0.9674
F2	0.4426	0.9972	22.62	-0.5453	0.995
F3	0.3444	0.9962	23.52	-1.766	0.997
F4	0.3341	0.9934	21.55	-1.45	0.9976
F5	0.405	0.9969	16.05	-0.465	0.9978
F6	0.3274	0.9921	17.86	-1.107	0.9849
F7	0.4226	0.9751	12.31	0.03701	0.9794
F8	1.72	0.9508	-38.92	30.65	0.9631

** Release exponent evaluated for < 60% released drug. r^2 is the correlation coefficient.*

3.5 Histopathological Study

A comparison was made between the most promising formulae based on *in vitro* release study results. The parameters of choice were the particle size, the encapsulation efficiency as well as the extent of drug released in the target area. It was found that F1 gave the smallest particle size (135 um), acceptable EE% (56 %) (Table 1). Moreover, F1 microspheres showed the highest % PR released after 24 hr (88.3 %) and only 20.6 % of the drug was released after 5 hr (Fig. 3A). Accordingly, F1 was selected as an optimum formula for colon specific delivery of PR and was subjected for further *in vivo* evaluation.

Fig. 4A showed the histological appearance of the colonic tissues of the normal control group. The normal colon shows normal histological structure of the mucosal lining epithelium and underlying lamina propria, sub mucosa, and muscularis. In case of colitis induced group that didn't receive treatment (group II) diffused necrosis in the mucosal layer with deformation in the underlying mucosal layer with edema and inflammatory cells infiltration in the sub mucosa, muscularis and serosa as seen in Fig. 4B. Figs. (4C and 4D) revealed diffuse inflammatory cells infiltration in the tissues of groups III and IV but no necrosis is defined in tissues treated with the selected microspheres formula (F1), on the other hand diffused necrosis is prominent in colonic tissues of group IV treated with the commercial tablets (Hostacortin-H®). It can be concluded that, treatment of TNBS-induced ulcerative colitis by the selected prednisolone microspheres formula may be more promising than that by the conventional prednisolone tablets.

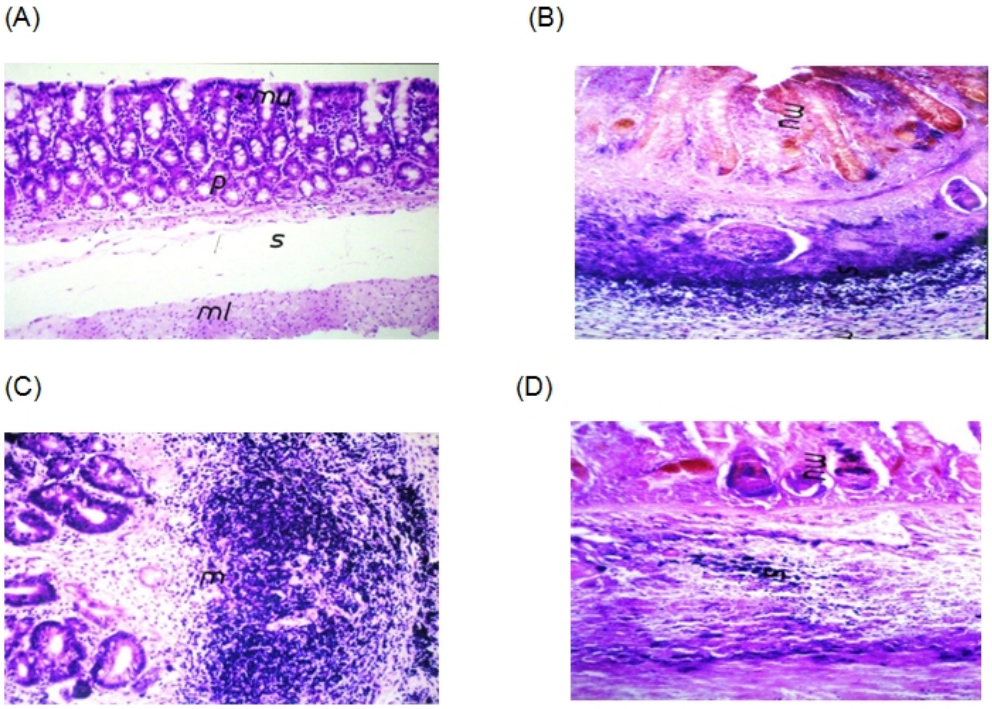

Fig. 4. Histological appearance of colonic tissues (A) normal control group, (B) TNBS-induced colitis group and didn't receive treatment, (C) Prednisolone microspheres (F1) treated group and (D) commercial tablets (Hostacortin-H®) treated group

3.6 *In vivo* Absorption Study

Fig. 5 showed the mean plasma concentration- time profiles of PR following oral administration of a single oral dose (5mg/kg) of free PR and F1 microspheres dispersions in 1% CMC to rats. Obviously, the two profiles were different regarding the T_{max} of the two treatments. The absorption of PR from the free drug dispersion was rapid; achieving peak plasma concentration of about 2.67 hr after dosing, showing that PR was immediately absorbed from rats GIT. However, the T_{max} of F1 microspheres was delayed to about 9.17 hr. Also a lag time of about 3 hr was obtained before plasma PR concentration could be detected after oral administration of PR microspheres.

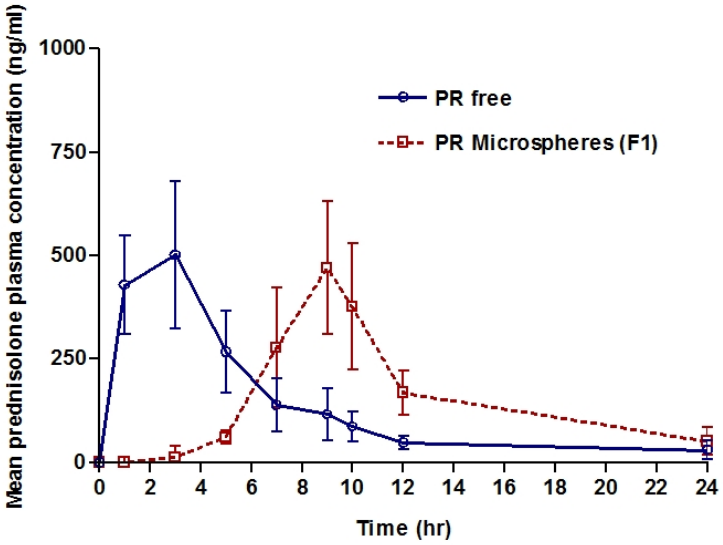

Fig. 5. Mean plasma concentration–time curves of prednisolone (PR) in rats (n=6) after administration of a single oral dose (5 mg/kg) of PR microspheres (F1) and free PR
F1:Ethylcellulose:Eudragit S100: 1% Span 80 1:1:0.16

The individual values of the pharmacokinetic parameters C_{max}, T_{max}, AUC_{0-24} and k_a were presented in Table 3. The mean values of C_{max} were (547.3 and 495.1 ng/ml), T_{max} (2.67 and 9.17 hr), K_a (1.137 and 0.388 hr^{-1}) and that of AUC_{0-24} were (3219.4 and 3638.8 ng. hr/ml), respectively after oral administration of free PR and F1 microspheres, respectively. By analyzing the parameters obtained from the different studied preparations through unpaired student- t test, no statistically significant difference was observed between the values of C_{max} (P > .05) as well as AUC_{0-24} (P >.05). However, there was significant difference between values of T_{max} (P = .03) and ka (P < .001) as presented in Table 3.

These results indicating that PR microspheres released the drug and the absorption takes places in the colonic region of the rats. In other words, regarding the extent of drug absorption in the target area, F1 gave a significantly (P<.001) higher absorption in comparison to free drug. The slower and delayed absorption of PR from F1 also indicated that the selected system released a significant amount of the drug at the colon; since colonic transit time was reported to be around 10 hr [31]. *In vivo* absorption studies revealed that the selected microspheres formula didn't release any significant amount of drug during passing the stomach. When the microspheres reached the large intestine, the drug release was

started in colonic fluids due to dissolution and erosion of microspheres made of combination of time- and pH- dependent polymers.

Table 3. The mean pharmacokinetic parameters of prednisolone after administration of a single oral dose (5mg/kg) of free drug and drug microspheres (F1) to rats (n=6)

Pharmacokinetic parameters	Mean (± SD, n=6)		*P* value
	Free prednisolone	Microspheres (F1)	
C_{max} (ng/ml)	547.3 ± 128.1	495.1 ± 177.4	0.572
T_{max} (hr)	2.67 ± 0.82	9.17 ± 0.41	0.0313*
AUC_{0-24} (ng.hr/ml)	3219.4 ± 744	3638.8 ± 1415.9	0.535
Ka (hr^{-1})	1.137 ± 0.155	0.388 ± 0.1156	0.0001*

* *Significant difference at $p < 0.05$. Values of parameters shown are mean ± SD (n = 6).*

4. CONCLUSION

The results in this investigation revealed that, the selected microspheres formula F1 consisted of 1:1:0.16 w/w/w EC: ES100: PR ratio and stabilized with 1% w/v span® 80 could deliver PR specifically to the colon. This approach may reduce frequent dosing and systemic side effects of the drug that may be produced by the conventional prednisolone tablet. The study has supplied us with brightening results concerning the therapeutic efficacy of colonic targeted microspheres formulated with a blend of time and pH- dependent polymers.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

COMPETING INTERESTS

The authors state no conflicts of interest and have received no funding for the research or in the preparation of this manuscript.

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