

Research Article

Controlled Release Formulation of Indomethacin Prepared With Bee Glue Extracts

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Abstract

Purpose: To prepare and evaluate new sustained release formulations of indomethacin based on extracts of propolis (bee glue).

Methods: Standardization of propolis (bee glue) extracts was performed by high performance liquid chromatography (HPLC) and determination of the values of fat and fixed oils. Several indomethacin capsule formulations (F1 - F18) containing varying amounts of chloroform (0.75 - 75 mg) and ethanol extracts (30 - 75 mg) of propolis were prepared. The dissolution rate of the formulations was evaluated by USP dissolution (rotating basket) method I and the release data subjected to various kinetic models. Probable interaction between the drug and propolis extracts was studied by differential scanning calorimetry (DSC).

Results: The results show that, although the release rate of formulations F1 - F7 did not show any significant difference ($p < 0.05$) compared to F18 as blank, the other formulations did. DSC results indicate that incorporation of propolis extract in the formulations lowered indomethacin melting point by between 5 and 30 °C, indicating interaction between the drug and the waxy extract. Kinetic analysis of the *in vitro* release data of the formulations showed that the best-fit drug release model varied with the drug:propolis extract ratio of the formulations.

Conclusion: Formulation F13 (with equal proportion of drug and bee glue extract) came out best from the dissolution test for indomethacin extended-release capsules as it exhibited zero order kinetics. This formulation is therefore suitable for further development as a matrix formulation for controlled release.

Keywords: Propolis (bee glue), Indomethacin, Controlled release, Zero order kinetics, Waxy materials

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INTRODUCTION

Propolis (bee glue) is the generic name for the resinous substance collected by honey bees from various plant sources. It is a strongly adhesive, resinous substance used by bees to seal holes in their hives and protect entry against intruders [1]. In general, propolis is composed of 50 % resin and vegetable balsam, 30 % wax, 10 % essential and aromatic oils, 5 % pollen and 5% various other substances, including organic debris.

Raw propolis is processed by water washing and dissolving in 95 % ethanol to remove wax and organic debris; the resulting mixture is often known as propolis tincture, "propolis balsam" or ethanol extract of propolis (EEP) [2]. The recorded use of propolis dates to as far as 300 BC and has continued as topical home remedies, as well as an ingredient in toothpaste and dental floss, and as a health-food/dietary supplement in various dosage forms - tablets, capsules, ampoules and syrups [3]. The United States Department of Agriculture describes propolis as follows: *"....a gum that is gathered by bees from various plants. It may vary in color from light yellow to dark brown; it may cause staining of the comb or frame and may be found in extracted honey"* [4].

Indomethacin is a well known non-steroidal anti inflammatory drug, administered by the oral route. In the last few decades, oral sustained-release dosage forms have been a focus of interest. Waxy materials have major applications in sustained release systems. Examples include hydrogenated oils [5], glyceryl stearate [6] and fatty alcohols [7]. Matrix delivery systems utilizing waxy materials usually employ a core of drug embedded in the wax or a compressed physical blend of drug and matrix forming agent. Several inert semi-solid excipients such as animal or vegetable waxes are available, and many have been considered for encapsulation [8].

Indomethacin microspheres coated with ethyl cellulose have been used to formulate sustained release suppositories [9] while gelatin-cellulose acetate phthalate microcapsules prepared by complex coacervation method have been employed to fabricate sustained release indomethacin tablets that effectively reduced rat stomach irritation compared to conventional indomethacin formulations [10]. Extended release lipophilic microspheres of indomethacin prepared with cetostearyl alcohol, stearyl alcohol, and cetyl alcohol in varying drug/lipid ratios fitted best to the Higuchi square root model for microspheres with the composition 1:4:1 drug-lipid ratio [14].

The objective of this study was to develop a new indomethacin controlled release solid dosage form using alcohol and chlorophorm extracts of propolis (bee glue) as matrices.

EXPERIMENTAL

Materials

Raw propolis was obtained from Sari (Mazandaran, Iran) bee keepers in autumn 2006 A sample (voucher no. 110 NTMRC) was kept at the herbarium of Traditional Medicine & Materia Medica Research Center (TMRC), Shaheed Beheshti University of Medical Sciences, Tehran, Iran. The propolis was kept in a desiccator in the dark until it was processed. Indomethacin, lactose and HPLC grade methanol were obtained from Merck (Darmstadt, Germany) while quercetin, kaempferol, naringenin, Chrysin and galangin were supplied by Extrasynthese (Genay, France).

Preparation propolis extracts

Ethanol extract of propolis (EEP) was then frozen at -10 °C, ground into powder in a mortar with a pestle. The powder was dispersed in 80 % ethanol (15 ml of ethanol/g of propolis) at 40 °C and shaken for 8 h. The mixture was filtered through Whatman filter

paper no. 1 and the extract concentrated to the past in a rotary evaporator under reduced pressure at 40 °C. The residue was redissolved in a minimal volume 0.5-1 ml of 96 % ethanol and kept at room temperature in the dark until use. The chloroform extract of propolis (CEP) was similarly obtained with chloroform used instead of ethanol.

Standardization of propolis extracts

The propolis extracts (EEP and CEP) were standardized using USP 25 procedures for the determination of parameters for fat and fixed oil, including acid, ester, hydroxyl, iodine and saponification values [15].

HPLC characterization of the extracts

Standardization was followed by HPLC characterization of the extracts using various flavonoids (quercetin, kaempferol, naringenin, chrysin and galangin) as standards [16]. The HPLC system (Knauer, Germany) consisted of a model K-1001 solvent delivery system equipped with a Rheodyn injection valve (20 µl sample loop inserted) and a UV-Vis spectrophotometric detector model K-2600 set at 360 nm (Knauer Associates, Germany). The analysis was performed using a ODS-C18 column (250 × 4.6 mm i.d., 5 µm particle size, Shim-Pack Vp-ODS) and the corresponding guard column (5 × 4.6 mm i.d., 5 µm particle size). All solvents were filtered and degassed before entering the column. The optimal conditions for the separation of five flavonoids were determined using Reverse phase (RP)-HPLC. The mobile phase was methanol: 30mM NaH₂PO₄ (40:60 v/v) adjusted to pH 3 while the mobile phase flow rate was 1.5 ml/min. All measurements were made at ambient temperature.

Preparation indomethacin capsule formulations

The desired amount of indomethacin was weighed and mixed with varying amounts of CEP and EEP dissolved in 0.5 ml chloroform

and ethanol, respectively (Table 1). In each case, the mixture, after evaporation of solvent, was passed through a sieve with aperture of 850 µm. Granules retained on the sieve were filled manually in capsule shells such that each capsule contained 75 mg indomethacin.

Table 1: Composition of 75 mg indomethacin capsule formulations. **Note:** F1 – F13 contained chloroform extract of propolis (CEP) while F14 – F17 contained the ethanol extract (EEP)

| Formulation code | Propolis extract (mg) | Lactose (mg) |
|------------------|-----------------------|--------------|
| F1 | 0.75 | 99.25 |
| F2 | 1.5 | 98.5 |
| F3 | 2.25 | 97.75 |
| F4 | 3 | 97 |
| F5 | 3.75 | 96.25 |
| F6 | 7.5 | 92.5 |
| F7 | 30 | 70 |
| F8 | 33.75 | 66.25 |
| F9 | 37.5 | 62.5 |
| F10 | 41.25 | 58.75 |
| F11 | 45 | 55 |
| F12 | 60 | 40 |
| F13 | 75 | 25 |
| F14 | 30 | 70 |
| F15 | 45 | 55 |
| F16 | 60 | 40 |
| F17 | 75 | 25 |
| F18 | 0 | 100 |

Drug release studies

In vitro release of indomethacin from the capsule formulations was determined using a standard USP 30 NF 25 dissolution test apparatus (rotating basket/method I, Erweka DT 80, Germany) at 100 rpm in 1 L of 0.01M hydrochloric acid (pH = 1.2) for 30 min and subsequently in 1 liter of phosphate buffer (pH 6.2) at 37 °C for 12 h. At predetermined time intervals, 5 ml aliquot samples were withdrawn from the dissolution medium, filtered through Whatman filter paper no. 1 and immediately replaced with the same

volume of fresh dissolution medium. Indomethacin content was determined spectrophotometrically (Genesys TM2, USA) at 318 nm. All determinations were in triplicate.

Differential scanning calorimetry (DSC) studies

Differential scanning calorimetric assessment of indomethacin, the individual extracts as well as selected blends of the drug and extracts were conducted in a Perkin Elmer (Germany) DSC apparatus using an accurate weight (~10 mg) of the sample in a loosely covered aluminum pan and heated from 50 to 300 °C at 10°C/min rate under nitrogen atmosphere. An empty loosely covered aluminum pan was used as the reference.

Kinetic analysis drug release data

To determine the release mechanisms of the formulations, the *in vitro* release data were subjected to various release kinetic models - zero-order, first-order, Higuchi, Korsmeyer-Peppas and Weibull with a view to obtaining the best fit.

Statistical analysis

The results obtained are expressed as mean ± standard deviation (SD). Student's t-test and one-way analysis of variance (ANOVA) were applied to check significant differences in drug release data between formulations using Sigma Plot 5 software. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Propolis standardization

Figure 1A shows the chromatogram (with retention times) of five flavonoid standards, namely, naringin (3.4 min), quercetin (5.3 min), kaemferol (7.2 min), chrysin (15.4 min) and galangine (17.8 min). Figure 1B shows the chromatogram of EEP with all the peaks

clearly resolved in base, except chrysin, while CEP chromatogram included quercetin, kaemferol and chrysin peaks (figure1-C).

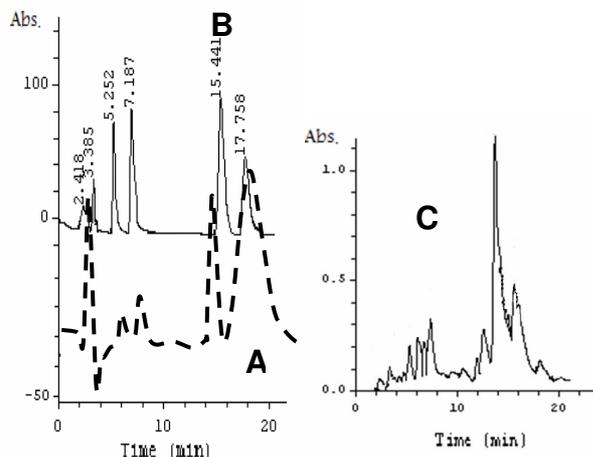


Figure 1: Representative HPLC chromatogram (A) of five flavonoid standards containing naringin (3.4 min.), quercetin (5.3 min.), kaemferol (7.3 min.), chrysin (15.4 min.) and galangine (17.8 min.); EEP chromatogram (B); and CEP chromatogram (C)

The results showed that due to existence of interferences at the ethanol and chloroform extract, peak overlapping was observed at 15.4 and 17.8 minute, respectively. Therefore, chrysin in the EEP and galangine in the CEP chromatogram are not detectable. Acid, ester, hydroxyl and saponification values for CEP were found 0.8, 78.54, 68.73 and 58.9 respectively.

Drug release

Indomethacin release from all the formulations was ≤ 4.5 % in 30 min in simulated gastric fluid (pH 1.2). The plots of the entire drug release data as a function of time, shown in Fig 2, indicate that there was no significant difference between formulations F1 – F7 and F18 which was the blank ($p > 0.05$), but the other formulations exhibited significant reduction in drug release compared with F18 ($p < 0.05$). Fig 2 shows the release profile of indomethacin with different percents of propolis. One way analysis of variance (ANOVA), indicated significant difference ($P < 0.05$) in release

profile of indomethacin, in capsules included propolis more than 33.75 mg per capsule.

F13, which represents a drug/CEP ratio of 1:1, produced the most pronounced decrease in drug release. Overall, the results show that using CEP produced significantly slower release of indomethacin than EEP.

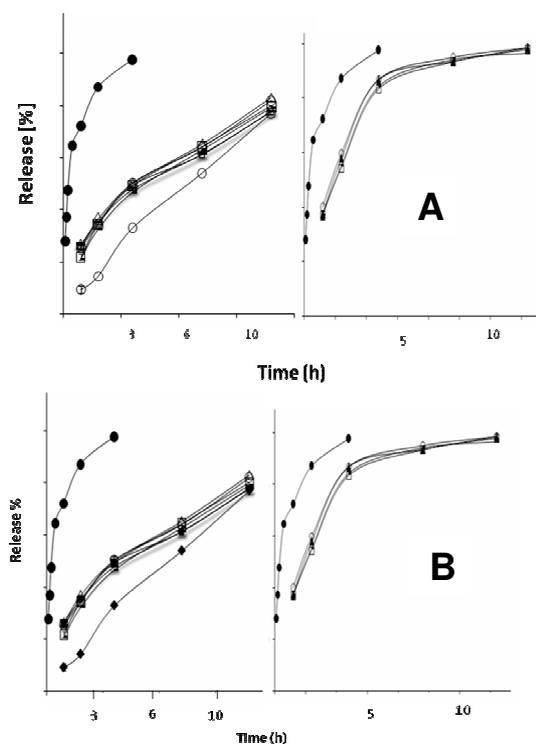


Figure 2: Dissolution profile ($n = 3$) of indomethacin capsule formulations containing propolis matrix; A = CEP-indomethacin formulations ($\square = F7$, $\diamond = F8$, $\blacktriangle = F9$, $\blacktriangledown = F10$, $\triangle = F11$, $\circ = F12$, $\Delta = F13$, $\bullet = F18$); B = EEP-indomethacin formulations ($\square = F14$, $\blacktriangle = F15$, $\circ = F16$, $\square = F17$, $\bullet = F18$ (see Table 1 for codes). Maximum release % in acidic medium <5%.

Drug release kinetics

Kinetic analysis of the *in vitro* release of the formulations indicate that the best-fit drug release model varied with the drug:propolis extract ratio of the formulations. All the derived kinetic parameters including rate constants are shown in Table 2. For the

Korsmeyer-Peppas model, diffusional exponent, n , is a factor which indicates the mechanism of the release. For instance $n:0.5$ for square root of time (~F8, F9-F11, and F12) and $n:1$ for zero order release (~F13). The values of $n>1.0$ indicates anomalous diffusion for F14-F17 formulations. The results in Table 2 show that the value of n increased when CEP content was increased (F7 to F13). F13 formulation fitted best to zero order and Korsmeyer-Peppas models.

Thermal characteristics

The DSC thermograms obtained are shown in Fig 3. They indicate a sharp endothermic peak at 165 °C for pure indomethacin (B) as well as a range of weak endothermic peaks from 100 to 200 °C and 30 to 100 °C for pure EEP (A) and CEP (C), respectively. These peaks can be attributed to melting transitions of the various lipids in the extract. Blending of propolis with indomethacin led to depression of the latter's melting point in all the formulations (D, E and F). The magnitude of these depressions were 5, 20 and 30 °C for F9, F13 and F17, respectively, which indicate probable interaction between the drug and propolis extracts.

DISCUSSION

In vitro dissolution test is an important tool for evaluating the quality of dosage forms obtained from various sources. It is also aids in assessing the capacity of a formulation to deliver the required active substance effectively to the patient.

The results indicate that indomethacin from the capsule formulations was pH-dependent, with < 5% of the drug released at acidic pH. The formulations showed a relatively rapid initial drug release during the first hour, followed by a slower release rate, except F13, which showed a relatively constant release rate. The initial rapid drug release may be due to the formation of a solid dispersion of the drug in the waxy material on

Table 2: Kinetic release parameters for indomethacin/propolis capsule formulations based on various release models

| Formulation Code | Zero-order model | | First-order model | | Higuchi model | | Korsemeyer-Peppas model | | | Weibull model | |
|------------------|------------------|-------|-------------------|-------|---------------|-------|-------------------------|-------|-------|---------------|-------|
| | K | r | K | r | K | r | K | r | n | K | r |
| F7 | 4.432 | 0.966 | 0.103 | 0.983 | 0.203 | 0.991 | 0.261 | 0.994 | 0.431 | 0.136 | 0.985 |
| F8 | 4.903 | 0.972 | 0.123 | 0.982 | 0.223 | 0.993 | 0.254 | 0.995 | 0.464 | 0.153 | 0.981 |
| F9 | 4.855 | 0.977 | 0.126 | 0.982 | 0.221 | 0.995 | 0.269 | 0.997 | 0.445 | 0.161 | 0.982 |
| F10 | 4.396 | 0.981 | 0.103 | 0.988 | 0.202 | 0.996 | 0.268 | 0.996 | 0.415 | 0.135 | 0.981 |
| F11 | 4.954 | 0.968 | 0.118 | 0.995 | 0.228 | 0.993 | 0.231 | 0.991 | 0.504 | 0.147 | 0.994 |
| F12 | 5.012 | 0.969 | 0.116 | 0.992 | 0.229 | 0.997 | 0.217 | 0.995 | 0.522 | 0.137 | 0.994 |
| F13 | 6.143 | 0.992 | 0.122 | 0.986 | 0.277 | 0.989 | 0.809 | 0.991 | 0.869 | 0.107 | 0.987 |
| F14 | 3.575 | 0.741 | 0.030 | 0.671 | 0.231 | 0.855 | 0.825 | 0.956 | 1.441 | 0.526 | 0.987 |
| F15 | 3.661 | 0.743 | 0.031 | 0.675 | 0.236 | 0.937 | 0.855 | 0.937 | 1.415 | 0.489 | 0.982 |
| F16 | 3.708 | 0.749 | 0.032 | 0.682 | 0.239 | 0.976 | 0.788 | 0.976 | 1.226 | 0.487 | 0.978 |
| F17 | 3.830 | 0.777 | 0.033 | 0.704 | 0.245 | 0.947 | 0.872 | 0.947 | 1.332 | 0.462 | 0.989 |

Note: r = correlation coefficient; K= (%/min): release rate constant; n = diffusional exponent

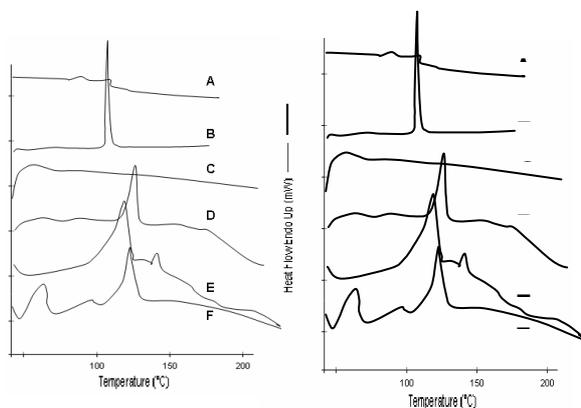


Fig 3: Differential scanning calorimetry (DSC) thermograms of (A) pure EEP, (B) pure indomethacin, (C) pure CEP, (D) F9 – drug/CEP (1:0.5), (E) F13 – drug/CEP (1:1), and (F) F17 – drug/EEP (1:1)

the surface of the granules; on the other hand, the drug present in the deeper interstices of the granules was released at a slower rate [17]. The slower but steadier release of F13 (in which the ratio of CEP to indomethacin was 1:1) is probably due to complete coating of the drug particles by CEP, would have hindered the penetration of the dissolution medium through the matrix.

As stated earlier, the mode of drug release from waxy matrices is usually by

erosion/diffusion of drugs [8]. There are different possible mechanisms by which this occurs. The first and most often encountered mechanism is drug diffusion through the outside layers of the matrix, and is known as “Fickian” release. The second mechanism is non-Fickian or anomalous transport while the third is “zero-order” release mechanism. All of the formulations exhibited retarded release of drug. However, increasing the ratio of propolis extracts resulted in further decrease in the drug release. Therefore, the dissolution medium slowly penetrated the granules, resulting in a slow release of the drug. In the present study, drug release from F13 exhibited zero order kinetics which means that although the formulation was a matrix system, the CEP coating around the drug particles functioned as a membrane system through which controlled drug diffusion occurred. Apparently, in formulations F1 to F12, there was insufficient matrix material to form a membrane system, and hence release did not follow zero order.

Drug release from non-swellable matrices is governed primarily by diffusion [17]. The rate of drug release from matrix systems decreases as a function of time because the diffusional path length for drug release decreases with time as the solvent front moves toward the center of the matrix. The

absence of positive deviation from linearity indicates that the drug is released primarily by diffusion and that erosion contributes negligibly. F13 formulation showed an optimal drug release properties as it closely approximated zero order. It seems that the dissolution of drug particles at the surface of other formulations allowed the establishment of channels in the matrix through which drug was released. Hence, release rate was higher in these formulations [18].

The values of the diffusional exponent, n , for the formulations was derived from the Korsmeyer-Peppas model (where n is a factor which indicates the mechanism of the release). For example, $n = 0.5$ (indicating diffusion-controlled drug release) and $n = 1.0$ (indicating swelling-controlled drug release). and $n > 1.0$ for anomalous diffusion. It is important to note that two extreme values for the exponent n , 0.5 and 1.0, are valid for only slab geometry. For spheres and cylinders, different values have been derived,

Another effective factor that influences drug release from a matrix system is the hydrophobicity of the matrix. The faster release of drugs formulated with the ethanol extract of propolis can be attributed in part to the higher hydrophobicity of the extract in which indomethacin (which itself is also a very hydrophobic drug) would readily disperse/dissolve in the solid state. In such circumstances, the drug would diffuse faster through the more hydrophobic matrix.

CONCLUSION

Our results show that CEP can be offered as a matrix material for sustained release of indomethacin and similarly hydrophobic drugs if used in a suitable proportion in relation to the drug. Such a delivery system would release drug by diffusion and/or erosion mechanisms. The advantage of propolis as a natural product is that it is more likely to be compatible with human body than synthetic matrix materials.

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