MICROENCAPSULATION OF ALLOPURINOL USING A FULLY IMPROVED NON-SOLVENT ADDITION TECHNIQUE AND A NOVEL BINARY BLEND BASED ON POLYVINYL CHLORIDE: FACTORIAL DESIGN APPLICATION

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A fully improved non-solvent addition coacervation-phase separation process which utilizes tetrahydrofurfuryl alcohol - cyclohexane: hexane (1:1 v/v) ratio as a solvent-non solvent pair, a novel binary blend comprising of polyvinyl chloride in combination with poloxamer 188 [a triblock copolymer of poly (ethylene oxide) - poly (propylene oxide)-poly (ethylene oxide)] as the wall material and ethylene-vinylacetate copolymer or polyisobutylene as coacervation-inducing agents has been developed for encapsulating allopurinol. The role of the coaddition of diocylphthalate as a plasticizer and poloxamer 188 as a complementary coating polymer to the polymer solution phase on the properties of the microcapsules was investigated. The results indicated that plasticized / blended microcapsules resulted in a great shift to a narrow particle size distribution and a significant reduction in the drug loss percentage and the drug release rate in comparison with unplasticized / unblended microcapsules or plasticized polyvinyl chloride microcapsules.

In the preparation of the plasticized / blended microcapsule formulations, a 2^4 full factorial design based on three independent variables viz.; polymers blend concentration, amount of allopurinol and the type of the coacervation-inducing agent was applied. The mean influences of these variables on the micrometric parameters (geometric mean particle diameter, span value and drug content) and dissolution properties of the microcapsules were characterized quantitatively and represented by predictor polynomial equations. The kinetic model according to the Rosin-Rimmler-Sperling-Bennett-Weillboll (RRSBW) distribution was applied for the linearization and parametric representation of the dissolution curves. The surface morphology of the microcapsules was examined by using scanning electron microscopy. The results revealed that using a lower polymers blend concentration level, a higher drug loading and ethylene-vinylacetate copolymer as a coacervation-inducing agent resulted in the formation of fairly spherical microcapsules of high monodispersity and better surface characteristics and extended the drug release period (τ: time at which 63.2 percent of the medicament dissolved = 6.052 h in simulated gastric fluid (S.G.F, pH 1.2) - 5.151 h in simulated intestinal fluid (S.I.F, pH 7.4). In contrast, polyisobutylene microcapsules prepared under the same experimental conditions were irregular, macroporous, and having the fastest release rates (τ = 4.48 h (S.G.F, pH 1.2) - 2.051 h (S.I.F, pH 7.4)). Studies of the drug release thermodynamics by the Arrhenius equation demonstrated clearly that the release of allopurinol is an energy-linked process by a single release mechanism. The formulation of microcapsules prepared using ethylene-vinylacetate copolymer into tablets, prolonged greatly the drug release.

INTRODUCTION

A great number of microencapsulation techniques are available for the formation of sustained-release microcapsules drug delivery systems.1-5 Although, the preparation of spherical microcapsules by the emulsion-solvent evaporation techniques such as w/o or w/o/w system has been achieved, a great disadvantage of these techniques is that the particle size and drug loading can be independently controlled.6,7

The non-solvent addition-phase separation method has gained an increased attention as one of the most common methods for preparing controlled-release delivery systems.8,9 Unfortunately, microcapsules obtained by this method are usually aggregated and irregular in shape.9,10 This may result in a low yield and inconsistent dissolution of the encapsulated drug as well as poor monodispersity, sphericity and rheological properties of the product that may obstacle the formulation of microcapsules into an appropriate dosage form such as tablets, capsules or sachets for further commercialization.10

One promising approach to develop a non-solvent addition technique for overcoming such inherent microencapsulation problems involves the proper selection of the polymer and its solvent / non-solvent system and the incorporation of a polymeric adjuvant or a complementary coating polymer by which the novelty of the system could be raised.
The application of polyvinyl chloride in drug formulation up till now has not gained much recognition. However, the polymer was utilized as a wall-forming material for the production of microcapsules and tablets in a number of reports.\textsuperscript{11-13} Das and Palchowdhury\textsuperscript{14} prepared polyvinyl chloride microcapsules containing sulphasuxiflorazol by a phase-separation coacervation method using chloroform and n-hexane as a solvent and non-solvent, respectively for obtaining controlled-drug release over a period of 8 h.

Substitution of the conventional polyvinyl chloride solvents such as tetrahydrofuran\textsuperscript{1} or chloroform\textsuperscript{14} due to its carcinogenic and toxological properties with edibies and drugs\textsuperscript{15} with a safer solvent such as tetraglycol (tetrahydrofuranyl alcohol (THFFA) or tetrahydrofurfuryl alcohol polyethylene glycol ether; glycofurol 75) which has no special toxicity properties and is used as a pharmaceutical solvent for parenteral products\textsuperscript{16} has not been reported.

The influence of plasticizers on the drug release characteristics of microcapsules and pellets was reported.\textsuperscript{10,17,18} However, a variety of plasticizers were used for the plasticization of vinyl chloride polymer for preparing monolithic polyvinyl chloride devices.\textsuperscript{19}

The non-ionic surfactants, pluronics: poly (ethylene oxide) - poly (propylene oxide) - poly (ethylene oxide) triblock copolymers are available in a wide range of molecular weights and hydrophilicity and thus provide further opportunities for modifying microcapsule characteristics.\textsuperscript{20,21} Huatan et al.\textsuperscript{21} reported that the inclusion of poloxamer 181 into poly (ε-caprolactone) matrix enhanced the particulate sphericity and regularity of protein-loaded microspheres. In addition, the hydrophilic surfactant coating; poloxamer 188 (pluronic F-68) retarded the release of diclofenac sodium from ethylcellulose microcapsules.\textsuperscript{22}

In the phase-separation technique by non-solvent addition, the formation of well-coated single microcapsules is facilitated by the presence of non-walling polymers such as butyl rubber, polyethylene and polyisobutylene.\textsuperscript{8,23} Sa et al.\textsuperscript{8} studied the effect of polyisobutylene (PIB) concentration and microcapsule size on the release of theophylline from ethylcellulose microcapsules and pointed out that PIB was found to influence the drug release profile to a great extent.

The use of ethylene-vinylacetate copolymer (EVA) as a coacervation-inducing agent in preparing theophylline-loaded ethylcellulose microcapsules by a phase-separation process based on temperature change was found to slower the drug release pattern.\textsuperscript{24} However, EVA copolymer has not hitherto been used in a microencapsulation system utilizing polyvinyl chloride as the coating polymer, nor is its role to successful formation of spherical microcapsules investigated.

Allopurinol as a uricosuric agent is mainly used for diseases like gout and leishmaniasis and to treat hyperuricaemia associated with chronic gout. Up to 90 percent of a dose of the drug is absorbed from the gastrointestinal tract after oral administration and its plasma half-life is about 1-3 hrs.\textsuperscript{25}

Direct use of this drug can have several side effects which include skin rashes, fever and other serious hypersensitivity reactions leading to renal and hepatic damage; in addition to the gastrointestinal tract disorders such as taste disturbance, nausea, vomiting, abdominal pain and diarrhoea.\textsuperscript{25}

Little is known about the microencapsulation of allopurinol which can reduce any side effects to a great extent. Prolonged release allopurinol microcapsules were prepared using a gelatin and formaldehyde polymer coating.\textsuperscript{26} A sustained-action allopurinol formulation (sigapurol CR) with a pH-controlled delivery has been marketed.\textsuperscript{27} Recently, Arabi et al.\textsuperscript{28} prepared allopurinol-loaded ethylcellulose microcapsules by a solvent evaporation method for a controlled release investigation of the drug.

The factorial design approach is applied for simultaneous determination of the effects of several factors and their interactions in complex systems.\textsuperscript{29} It offers a good degree of accuracy for screening purposes as factor effectiveness can be expressed with a mathematical model which explains the influence numerically.\textsuperscript{29-31} The application of factorial design to tablet formulations,\textsuperscript{32,33} microcapsules\textsuperscript{30,34} and tableted microcapsules\textsuperscript{31} has been investigated.
In this study, a fully-improved non-solvent addition phase-separation process which employed a novel binary blend based on polyvinyl chloride, as the coating material, tetrahydrofurfuryl alcohol : cyclohexane : hexane (1:1 v/v ratio) as a solvent - non-solvent pair and ethylene-vinylacetate copolymer or polyisobutylene as a coacervation-inducing agent was developed. The purpose of the present study was: (a) to investigate by the adopted technique, the production of sustained-release microcapsules for allopurinol and to clarify the effectiveness of dioctylphthalate as a plasticizer and poloxamer 188 as a complementary coating polymer in the blend in improving the quality of microcapsules and (b) to utilize a 2^3 full factorial design for estimating quantitatively the effect of different preparation variables viz., polymers blend concentration, amount of allopurinol and the type of the coacervation-inducing agent on the micromeric and dissolution characteristics as well as the surface morphology of the plasticized/blended microcapsules aiming at selecting a set of optimal experimental conditions for the production of sustained-release microcapsules having good sphericity and monodispersity. The formulation of the produced microcapsules into tablets was also investigated for further retardation of the drug release process.

MATERIALS AND METHODS

Materials

Allopurinol (Sigma Chemical Co. St. Louis, MO, USA), polyvinyl chloride (PVC, MW 37400), dioctylphthalate (DOP) (di (2-ethylhexyl) phthalate) (Wako Pure Chemicals, Tokyo, Japan), ethylene-vinylacetate copolymer (EVA) (vinyl acetate content: 33%), polyisobutylene (PIB, MW 380,000) (Aldrich Chemical Co., Inc., Milwaukee, Wisconsin, USA), poloxamer 188 (PLX 188) (ICI surfactants, Cleveland, England), tetrahydrofurfuryl alcohol (THFFA) (BDH Chemicals, Ltd, Poole, England), n-hexane and cyclohexane (Advic, El-Nasr Chemical Co., Cairo, Egypt), according to the methods of Prolabo, Paris, France) and Avicel PH 101 (Fluka AG, CH-9470 Buchs, Switzerland). All other chemicals were of reagent grade and were used as received.

Methods

Factorial design experiments

The effects of the polymers blend concentration (A), amount of allopurinol (B) and the type of coacervation-inducing agent (C) on the microcapsule properties were performed with a 2^3 full factorial design experiments. The independent variables with their levels and the calculation matrix for the 2^3 factorial design investigated in the preparation of allopurinol microcapsules are presented in Tables 2a and 2b, respectively. The dependent variables are the geometric mean particle diameter (dg), span value (S), drug content percent and τ (the time at which 63.2 percent of the medicament dissolved) and β (shape parameter) values of the dissolution kinetic (RRSBW distribution). The response data obtained from the 2^3 experiments for each combination of factors are given in Tables 4 and 5. The mean effects of the individual variables (A, B and C) and their interactions between the three variables in the factorial design of experiments can be calculated using the contrast coefficients method. Fitting a multiple linear regression equation to a 2^3 factorial design gave a predictor equation which was a first order polynomial, having the form:

\[ Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1x_2 + b_5x_1x_3 + b_6x_2x_3 + b_7x_1x_2x_3 \]  

(1)

where Y: level of a given response (dependent variable); b: regression coefficients for the first order polynomial and x: level of the independent variable.

Data were analyzed using analysis of variance (ANOVA) and regression coefficients were calculated for factors having significant effect at p < 0.01 and/or 0.05.

Preparation of microcapsules

Polyvinyl chloride / poloxamer 188 microcapsules containing allopurinol were prepared by a fully developed non-solvent addition coacervation-phase separation process
employing tetrahydrofurfuryl alcohol (THFFA) as the polymer solvent and a mixture of cyclohexane: n-hexane at 1:1 v/v ratio as the non-solvent in the presence of an appropriate concentration (3% w/w) of ethylene-vinyl acetate co-polymer (EVA) or polyisobutylene (PIB) as a coacervation-inducing agent. A series of polyvinyl chloride / poloxamer 188 microcapsules were prepared using different preparation conditions according to the following basic procedure: polyvinyl chloride (0.454 gm) was dissolved in 10 ml of THFFA on heating. Diocetylphthalate as a plasticizer at 32.3% w/w concentration (based on total coat weight) and poloxamer 188 dissolved at 10% w/w concentration (based on total polymers weight) in dichloromethane were added to the polymer solution. The required amount of allopurinol was uniformly dispersed in the polymer solution. After stirring the system thoroughly with a mechanical stirrer (Wheaton Instruments, North Tenth Street, Millville, N.J., USA) at a rate of 200 rpm and at room temperature (25°C±0.5). The non-solvent solution containing span 85 was added dropwise at a constant rate of 100 drop/min to precipitate the coat around the drug particles and produce the microcapsules.

Ten minutes after the formation process was completed, the microcapsules sank and were separated by decantation and rinsed twice with 50 ml portions of chilled n-hexane for 10 h. in a refrigerator to rigidize the microcapsules. The microcapsules were collected by filtration, washed twice with cyclohexane (50 ml) to remove any EVA or PIB adsorbed at the microcapsule interface and then air-dried at room temperature for 48 h in open air. The dried microcapsules were separated into different size fractions using a nest of standard sieves.

For comparision, microcapsules in the absence of dioctylphthalate and/or poloxamer 188 were prepared by the same procedure described above.

The effects of the following preparation variables on the microcapsule properties were investigated: polymers blend concentration, amount of allopurinol and the type of coacervation-inducing agent. The influence of pH of the dissolution media and microcapsules size on the release characteristics of microcapsules was also studied.

**Preparation of tabletted microcapsules**

Tablets were made by direct compression of the dried microcapsules having an average particle size of 427.5 μm and prepared by using a polymers blend concentration of 5% w/v, allopurinol amount of 0.454 gm and ethylene-vinylacetate copolymer as a coacervation-inducing agent (treatment combination b, Table 3). Each tablet formulation contained an amount of microcapsules equivalent to 50 mg drug, 77% w/w Avicel PH 101 as a filler and disintegrating agent and 2% w/w magnesium stearate as a lubricant. The compression was carried out with an IR carver hydraulic press (Model 3912, Carver, Inc. St. Morris, Wabash, USA) under a compression pressure of 2.2 tons/inch² for 15 seconds followed by a quick release to produce flat-faced tablets of 13 mm diameter.

**Linearization of the particle size distribution**

The particle size distribution was characterized by the log-normal distribution equation where the relation between the cumulative frequency, P and the particle diameter, d, expressed by:

\[ P = \frac{100}{\sqrt{2\pi} \sigma_g} \int_{\log d}^{\log d} \exp \left( -\frac{1}{2} \left( \frac{\log d - \log d_g}{\sigma_g} \right)^2 \right) d(\log d) \]

(2)

The particle size log-normal distribution is then characterized by two parameters; the geometric mean particle diameter, d_ge (the particle size pertaining to P= 50 percent throughfall) and the geometric standard deviation, \( \sigma_g \).

**Determination of the drug content**

An accurately weighed amount of microcapsules (50 mg) were crushed in mortar using a pestle. Allopurinol was extracted with simulated intestinal fluid (pH 7.4). The solution was filtered and allopurinol was assayed spectrophotometrically at 250 nm using the same medium as a blank.
**In-vitro release studies**

The release experiments were conducted using the USP XXII rotating paddle dissolution apparatus (Model Dt-06, Erweka (F.R.G.)) at 50 rpm. Fifty milligrams of microcapsules were accurately weighed and added to 250 ml of enzymes free-dissolution medium (simulated gastric fluid (S.G.F): HCL/NaCl solution, pH 1.2 or simulated intestinal fluid (S.I.F.): KH₂PO₄/NaOH buffer, pH 7.4) containing 0.02% w/v tween 80 and maintained at 37°C ± 0.2. At appropriate time intervals, 5 ml samples were withdrawn and replaced by an equivalent volume of the dissolution medium kept at 37°C. The dissolution patterns of the drug powder and tablet formulations were similarly studied. A series of experiments was also conducted in which the in-vitro release of allopurinol from the microcapsules (treatment combinations b, bc: Table 3) prepared with different coacervation-inducing agents was carried out at additional three selected temperatures; 25, 45, 50°C. The concentration of allopurinol was determined spectrophotometrically at 250 nm. All dissolution studies were run in duplicate.

**Linearization and kinetic evaluation of the dissolution rate constants**

All of the results thus obtained were evaluated kinetically by zero-order, first-order, Higuchi release and RRSBW (Rosin-Rimmeli-Sperling-Bennett-Wellbull) dissolution kinetics. The release rate constants, correlation coefficients (r) and determination coefficients (r²) were calculated for each of kinetic model. The RRSBW distribution in the logarithmic form is shown in equation 3:

\[
\ln \ln \frac{1}{1 - m/m_\infty} = -\beta \ln (t - t_o) - \beta \ln \tau
\]

where m is the amount of medicament dissolved at time t; m_\infty is the amount of medicament dissolved at time \infty; t is time; t_o is lag time; \tau is the time at which 63.2 percent of the medicament dissolved and \beta (slope) is the shape parameter of the dissolution curve. In order to indicate the influence of factors on the dissolution kinetics, the \tau and \beta parameters were selected (Table 5). The change in the shape parameter (\beta) means that when \beta > 1, it reports upon the parallel moving courses in addition to diffusion, disintegration and erosion.²⁴,³⁶

**Scanning electron microscopy**

The surface morphology of microcapsules was examined using a JEOL scanning electron microscope (JSM-5200, Japan) as follows: microcapsules were coated with gold in a vacuum for 10 min. at 60 milliamperes by using SPI Sputter™ coating unit (SPI supplies, division of structure probe, Inc., West Chester, PA, USA). Scanning electron micrographs were taken at 15 kV.

**RESULTS**

**Development of polyvinyl chloride microcapsules for allopurinol**

In the initial study, the influences of the use of dioctyl phthalate (DOP) as a plasticizer and poloxamer 188 (PLX 188) as a complementary coating polymer in the microencapsulation process on the micromeritic properties of allopurinol microcapsules prepared by using a polymer concentration of 5% w/v at an initial drug amount of 0.227 gm and ethylene-vinylacetate (EVA) copolymer as a coacervation-inducing agent were investigated (Table 1).

The results indicated that the percentage of drug loss for microcapsules M1 (prepared without DOP and PLX 188) and microcapsules coded M2 (which contain 35.5% w/v DOP based on total coat weight) was estimated to be 37.33 and 36.18, respectively. On the contrary, microcapsules M3, M4 and M5 (which contain DOP and PLX 188) exhibited a lower drug loss of about 1 to 12.08 percent depending on the preparation conditions. This signifies that there was an appreciable degree of drug incorporation (encapsulation efficiencies of 88 to 99%) meaning that the exercise of encapsulating allopurinol was successful.
Table 1: Effect of diocetylphthalate as a plasticizer and poloxamer 188 as an added complementary coating polymer on the micromeric properties of allopurinol-loaded polyvinyl chloride microcapsules.

<table>
<thead>
<tr>
<th>Code</th>
<th>Diocetylphthalate (% w/w)</th>
<th>Poloxamer 188 (% w/w)</th>
<th>dg (µm)</th>
<th>σg</th>
<th>S</th>
<th>Theoretical drug content (%)</th>
<th>Measured drug content (%)</th>
<th>Drug loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>-</td>
<td>-</td>
<td>484.17</td>
<td>1.190</td>
<td>0.4699</td>
<td>33.33</td>
<td>20.89</td>
<td>37.33</td>
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<tr>
<td>M2</td>
<td>35.5</td>
<td>-</td>
<td>638.26</td>
<td>1.189</td>
<td>0.4700</td>
<td>24.38</td>
<td>15.56</td>
<td>36.18</td>
</tr>
<tr>
<td>M3</td>
<td>33.2</td>
<td>10.0</td>
<td>473.15</td>
<td>1.110</td>
<td>0.2740</td>
<td>23.14</td>
<td>20.45</td>
<td>11.63</td>
</tr>
<tr>
<td>M4</td>
<td>31.13</td>
<td>18.08</td>
<td>412.10</td>
<td>1.175</td>
<td>0.4223</td>
<td>22.02</td>
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<td>1.000</td>
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<tr>
<td>M5(b)</td>
<td>31.13</td>
<td>18.08</td>
<td>457.10</td>
<td>1.210</td>
<td>0.4830</td>
<td>22.02</td>
<td>19.36</td>
<td>12.08</td>
</tr>
</tbody>
</table>

Microencapsulation conditions: polymer concentration: 5% w/v, amount of allopurinol: 0.227 gm, ethylene-vinylacetate copolymer as a coacervation-inducing agent and a non-solvent addition rate of 100 drop/min.

dg: geometric mean particle diameter (µm), σg: geometric standard deviation, S: span value,

\[ S = \frac{D_{95\%} - D_{10\%}}{D_{50\%}} \]

where \( D_{95\%}, D_{50\%} \) and \( D_{10\%} \) are the diameters which give the corresponding particle percentage, respectively.

\( a^o\) Drug loss (%) = \( \frac{\text{Theoretical drug content} - \text{Measured drug content}}{\text{Theoretical drug content}} \times 100 \)

\( b)\) Microcapsules were prepared using a non-solvent addition rate of 60 drop/min.

As can be depicted also from Table 1, microcapsules M3 (which have 33.2% w/w DOP and 10% w/w PLX 188 based on total polymers weight) had a lower geometric mean particle diameter (dg: 473.15 µm) and the lowest geometric standard deviation (σg: 1.11) and span value (measure of monodispersity) of (S: 0.247). However, microcapsules M1 (No DOP) and M2 (35.5% w/w DOP) exhibited the highest geometric mean particle diameters (dg’s: 484.17 and 638.26 µm, respectively) and their σg and S values were increased by about 7.2 and 41.7 percent, respectively in comparison with microcapsules M3. Microcapsules M4 (non-solvent addition rate of 100 drop/min and 18.08% w/w PLX 188) had the lowest dg (dg: 412.10 µm) and a drug loss of one percent, whereas, code M5 (non-solvent addition rate of 60 drop/min and 18.08% w/w PLX 188) exhibited the highest σg and S values (1.21 and 0.483, respectively).

Figure 1 shows the size distribution of various allopurinol-loaded polyvinyl chloride microcapsules prepared with different preparation conditions. Generally, the particle size of microcapsules varied from approximately 150 to 710 µm with a peak value between 302.5 to 550 µm. In addition, the results revealed that the particle size of plasticized polyvinyl chloride / poloxamer 188 composite microcapsules decreased significantly with increasing amount of PLX 188 in the polymers blend from 10% w/w (code M3, frequency of smaller microcapsules of an average size of 302.5 µm was 11.57 percent) to 18.08% w/w (code M4; frequency of smaller microcapsules of an average size of 302.5 µm was 52.33 percent). Conversely, using DOP in the microcapsules preparation without PLX 188 (code M2) resulted in the highest frequency (37.79 percent) of larger microcapsules having an average size of 550 µm.

The in-vitro release profiles of allopurinol from microcapsules prepared using various preparation conditions were conducted in simulated intestinal fluid (S.I.F., pH 7.4) (Figs. 2 and 3). It is evident that microcapsules M2 (which have 35.5% w/w DOP and a drug content of 15.56% w/w) exhibited a higher release rate \( (K = 7.33 \times 10^{-1} \text{ h}^{-1}) \) than microcapsules M1 prepared without DOP and
Figure 2: Effect of poloxamer 188 on the in vitro release of diclofenac from poloxamer 188 \& diclofenac-loaded microparticles.

Figure 3: Effect of poloxamer 188 on the in vitro release of diclofenac from poloxamer 188 \& diclofenac-loaded microparticles.
having a drug content of 20.89% w/w (K₁ = 4.055x10⁻¹ h⁻¹) (Fig. 2).

The release patterns of allopurinol from polyvinyl chloride / poloxamer 188 composite microcapsules revealed that, by using PLX 188 at 10% w/w concentration as an added complementary coating material, the microcapsules M3 having a drug content of 20.45% w/w would achieve good sustained release properties (K₁ = 3.10x10⁻¹ h⁻¹) when compared to other formulations prepared with PLX 188 at 18.08% w/w concentration (K₁'s = 3.73x10⁻¹ - 5.23x10⁻¹ h⁻¹) (Fig. 3).

Evaluation of the factorial design results of the micromeritic studies of allopurinol-loaded polyvinyl chloride / poloxamer 188 microcapsules

The factorial design model was applied to the evaluation of the micromeritic and dissolution characteristics of allopurinol microcapsules. The factors studied are summarized in Table 2a, which contains the lower (-) and the higher (+) levels of the variable parameters. The calculation matrix of the variables studied and their interactions in the factorial design model are represented in Table 2b. The effect was calculated from the change in the micromeritic properties, i.e. the log-normal distribution parameters (geometric mean particle diameter (dg), geometric standard deviation (σg), span value (S) and the percentage of drug content) of the resulting microcapsules.

Table 3 shows the effects of the polymers blend concentration (A), amount of allopurinol (B) and the type of the coacervation-inducing agent (C) and their interactions between the three variables in the factorial design of experiments on the micromeritic properties of allopurinol microcapsules. It can be seen from the data that the variance (geometric standard deviation, σg) showed the greatest deviance in treatment combinations (1) and (ac) whose their σg's values were 1.11 and 1.19, respectively (Table 3). The experimental particle size average pertaining to 50 percent throughfall (dg), average span value (S) and the mean percentage of drug content are 428.4 μm, 0.391 and 25.27 percent respectively. These values can be obtained from experiments with basic levels of all the factors. The mean diameter of microcapsules depended to a great extent on the type of the coacervation-inducing agent (Table 3). Thus, using polyisobutylene (PIB) as a coacervation-inducing agent resulted in decreasing the microcapsule size (dg: 384.59-407.38 μm) and increasing the span value (S: 0.436-0.4554) in comparison with EVA (dg: 389.05-489.78, S: 0.274-0.3939).

The payload was not greatly changed for the whole series of the same initial drug amount or microcapsule size, irrespective of the type of the coacervation-inducing agent (Table 3). Generally, a higher encapsulation efficiencies (85.36-99%) were obtained due to a decrease in the drug loss (1.11-14.64% depending on the preparation conditions) (Table 3).

Figure 4 shows the influence of the independent variables (polymers blend concentration, amount of allopurinol and type of coacervation-inducing agent) of the factorial design on the size distribution of allopurinol-loaded polyvinyl chloride / poloxamer 188 microcapsules. It appeared that microcapsules prepared with PIB had a broader particle size distributions. The highest frequency (45.64-65.35 percent) for smaller microcapsules having an average size of 302.5 μm were also observed, whereas the use of EVA as a coacervation-inducing agent was noted to produce narrow and symmetrical particle size distributions about the arithmetic mean with the highest frequency (80.95-72.38 percent) of microcapsules (treatment combinations (1), b respectively) having an average size of 427.5 μm.

The analysis of results was evaluated with analysis of variance (ANOVA) and the significance of the observed effects was tested with the F test at the 95 and 99 percent probability levels (Tables 4 and 5).

Based on the mean responses illustrated in Table 4, the resultant equations of the fitted first-order polynomial model obtained as a result of the 2³ factorial design for the micromeritic parameters of allopurinol microcapsules are:

\[ Y_i = 428.4 - 16.023X_1 - 10.7X_2 - 27.883X_3 - 14.858X_1X_2 + 9.16X_1X_3 + 6.168X_2X_3 + 10.33X_1X_2X_3 \]  

(4)
Table 2: (a) A 2³ factorial design parameters (independent variables) and experimental conditions; (b) calculation matrix of the 2³ factorial design.

(a)

<table>
<thead>
<tr>
<th>Factors (independent variables)</th>
<th>Level</th>
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<tr>
<td></td>
<td>Low (-1)</td>
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<tr>
<td>A: polymers blend concentration (% w/v)</td>
<td>5</td>
</tr>
<tr>
<td>B: amount of allopurinol (gm)</td>
<td>0.227</td>
</tr>
<tr>
<td>C: type of coacervation-inducing agent</td>
<td>EVA</td>
</tr>
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</table>

EVA: ethylene-vinylacetate copolymer, PIB: polyisobutylene.

(b)

<table>
<thead>
<tr>
<th>Treatment combination</th>
<th>X₀</th>
<th>X₁</th>
<th>X₂</th>
<th>X₁X₂</th>
<th>X₃</th>
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<td>+1</td>
<td>+1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>b</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>ab</td>
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<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>c</td>
<td>+1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>ac</td>
<td>+1</td>
<td>+1</td>
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<td>-1</td>
</tr>
<tr>
<td>bc</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
<td>1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>abc</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
</tr>
</tbody>
</table>

* -1: factor at low level, +1: factor at high level, X₀: total.

Fig. 4: Particle size distribution plots of allopurinol-loaded polyvinyl chloride/polyolamer 188 microcapsules prepared by using EVA copolymer (A) and PIB (B) as coacervation-inducing agents. Microencapsulation conditions: 33.2% w/w dioctylphthalate, 10% w/w poloxamer 188 and a non-solvent addition rate of 100 drop/min. (See Table 3 for treatment combinations key).
<table>
<thead>
<tr>
<th>Treatment combination</th>
<th>Polymers blend concentration (% w/v)</th>
<th>Amount of allopurinol (gm)</th>
<th>Coacervation-inducing agenta</th>
<th>dg (μm)</th>
<th>σg</th>
<th>S</th>
<th>Theoretical drug content (%)</th>
<th>Measured drug content (%)</th>
<th>Drug lossb (%)</th>
<th>Fraction size (μm)</th>
<th>Fraction size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>5.0</td>
<td>0.227</td>
<td>EVA</td>
<td>473.15</td>
<td>1.110</td>
<td>0.2740</td>
<td>23.14</td>
<td>21.54</td>
<td>6.914</td>
<td>250-355</td>
<td>355-500</td>
</tr>
<tr>
<td>a</td>
<td>7.5</td>
<td>0.227</td>
<td>EVA</td>
<td>473.15</td>
<td>1.135</td>
<td>0.3374</td>
<td>19.06</td>
<td>16.27</td>
<td>14.640</td>
<td>10,120</td>
<td>11.120</td>
</tr>
<tr>
<td>b</td>
<td>5.0</td>
<td>0.454</td>
<td>EVA</td>
<td>489.78</td>
<td>1.142</td>
<td>0.3374</td>
<td>37.58</td>
<td>34.88</td>
<td>7.180</td>
<td>250-355</td>
<td>355-500</td>
</tr>
<tr>
<td>ab</td>
<td>7.5</td>
<td>0.454</td>
<td>EVA</td>
<td>389.05</td>
<td>1.162</td>
<td>0.3939</td>
<td>32.01</td>
<td>27.46</td>
<td>14.220</td>
<td>250-355</td>
<td>355-500</td>
</tr>
<tr>
<td>c</td>
<td>5.0</td>
<td>0.227</td>
<td>PIB</td>
<td>407.38</td>
<td>1.182</td>
<td>0.4502</td>
<td>23.14</td>
<td>22.64</td>
<td>2.161</td>
<td>250-355</td>
<td>355-500</td>
</tr>
<tr>
<td>ac</td>
<td>7.5</td>
<td>0.227</td>
<td>PIB</td>
<td>402.72</td>
<td>1.190</td>
<td>0.4554</td>
<td>19.06</td>
<td>17.21</td>
<td>9.679</td>
<td>250-355</td>
<td>355-500</td>
</tr>
<tr>
<td>bc</td>
<td>5.0</td>
<td>0.454</td>
<td>PIB</td>
<td>407.38</td>
<td>1.188</td>
<td>0.4410</td>
<td>37.58</td>
<td>33.27</td>
<td>11.480</td>
<td>250-355</td>
<td>355-500</td>
</tr>
<tr>
<td>abc</td>
<td>7.5</td>
<td>0.454</td>
<td>PIB</td>
<td>384.59</td>
<td>1.175</td>
<td>0.4360</td>
<td>32.01</td>
<td>28.00</td>
<td>12.530</td>
<td>250-355</td>
<td>355-500</td>
</tr>
</tbody>
</table>

Microencapsulation conditions: 33.2% w/w dioctylphthalate, 10% w/w poloxamer 188, and a non-solvent addition rate of 100 drop/min.

dg: geometric mean particle diameter (μm), σg: geometric standard deviation, S: span value, $S = \frac{D_{90\%} - D_{10\%}}{D_{50\%}}$, where $D_{90\%}$, $D_{50\%}$ and $D_{10\%}$ are the diameters which give the corresponding particle percentage, respectively.

a) EVA: ethylene-vinylacetate copolymer, PIB: polyisobutylene.

b) Drug loss (%) = \[ \frac{\text{Theoretical drug content} - \text{Measured drug content}}{\text{Theoretical drug content}} \times 100 \]
### Table 4: Results and Analysis of Variance of a 2 Factorial Design Experiments for the Effect of Polymers Blend Concentration, Amount of Chitosan-loaded Polyelectrolyte Chitosan/Poloxamer 188 Microcapsules.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean</th>
<th>d.f.</th>
<th>Mean</th>
<th>d.f.</th>
<th>Mean</th>
<th>d.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymers Blend</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount of Chitosan-loaded Polyelectrolyte Chitosan/Poloxamer 188 Microcapsules</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance level based on 1 d.f. (p > 0.05) for 0.05-level. EMS: Error mean square.
Table 5: Results and analysis of variance of a $2^3$ factorial design experiments for the effect of polymers blend concentration, amount of allopurinol and type of coacervation-inducing agent on the $\tau$ and $\beta$ values of the RRSBW distribution kinetics.

### A. In S.G.F. (pH 1.2).

<table>
<thead>
<tr>
<th>Treatment combination</th>
<th>Response $\tau$ (min)</th>
<th>Mean effect</th>
<th>d.f</th>
<th>Mean square</th>
<th>F</th>
<th>Response $\beta$</th>
<th>Mean effect</th>
<th>d.f</th>
<th>Mean square (x10^2)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>380.19</td>
<td>-89.71</td>
<td>1</td>
<td>16096.67</td>
<td>670.7''</td>
<td>1.0196</td>
<td>-</td>
<td>1</td>
<td>2.843</td>
<td>3.18</td>
</tr>
<tr>
<td>a</td>
<td>223.87</td>
<td>-2.533</td>
<td>1</td>
<td>12.827</td>
<td>0.534</td>
<td>0.8997</td>
<td>-0.0377</td>
<td>1</td>
<td>6.294</td>
<td>7.03</td>
</tr>
<tr>
<td>b</td>
<td>363.08</td>
<td>2.108</td>
<td>1</td>
<td>8.383</td>
<td>-</td>
<td>0.8255</td>
<td>0.0281</td>
<td>1</td>
<td>1.580</td>
<td>-</td>
</tr>
<tr>
<td>ab</td>
<td>331.13</td>
<td>107.04</td>
<td>1</td>
<td>22916.2</td>
<td>954.84''</td>
<td>0.8871</td>
<td>0.0698</td>
<td>1</td>
<td>9.73</td>
<td>10.87</td>
</tr>
<tr>
<td>c</td>
<td>251.19</td>
<td>4.423</td>
<td>1</td>
<td>39.117</td>
<td>-</td>
<td>0.8854</td>
<td>0.0744</td>
<td>1</td>
<td>11.1</td>
<td>12.36</td>
</tr>
<tr>
<td>ac</td>
<td>223.87</td>
<td>-42.54</td>
<td>1</td>
<td>3619.73</td>
<td>150.82''</td>
<td>1.043</td>
<td>0.1382</td>
<td>1</td>
<td>38.20</td>
<td>42.68*</td>
</tr>
<tr>
<td>bc</td>
<td>125.89</td>
<td>-60.08</td>
<td>2</td>
<td>7218.61</td>
<td>300.78''</td>
<td>1.118</td>
<td>0.0103</td>
<td>1</td>
<td>0.210</td>
<td>-</td>
</tr>
<tr>
<td>abc</td>
<td></td>
<td></td>
<td></td>
<td>EMS=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

### B. In S.I.F. (pH 7.4)

<table>
<thead>
<tr>
<th>Treatment combination</th>
<th>Response $\tau$ (min)</th>
<th>Mean effect</th>
<th>d.f</th>
<th>Mean square</th>
<th>F</th>
<th>Response $\beta$</th>
<th>Mean effect</th>
<th>d.f</th>
<th>Mean square (x10^2)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>281.84</td>
<td>-64.69</td>
<td>1</td>
<td>8369.60</td>
<td>63.08''</td>
<td>1.058</td>
<td>-0.1393</td>
<td>1</td>
<td>38.81</td>
<td>12.53*</td>
</tr>
<tr>
<td>a</td>
<td>223.87</td>
<td>-20.71</td>
<td>1</td>
<td>857.81</td>
<td>6.47</td>
<td>1.106</td>
<td>-0.0029</td>
<td>1</td>
<td>0.0174</td>
<td>0.00562</td>
</tr>
<tr>
<td>b</td>
<td>177.83</td>
<td>-34.31</td>
<td>1</td>
<td>2353.67</td>
<td>17.74</td>
<td>1.0054</td>
<td>-0.0395</td>
<td>1</td>
<td>3.113</td>
<td>-</td>
</tr>
<tr>
<td>ab</td>
<td>123.03</td>
<td>-142.51</td>
<td>1</td>
<td>40618.2</td>
<td>306.12''</td>
<td>1.096</td>
<td>-0.0083</td>
<td>1</td>
<td>0.1378</td>
<td>0.0445</td>
</tr>
<tr>
<td>ac</td>
<td>120.23</td>
<td>29.89</td>
<td>1</td>
<td>1</td>
<td>13.47</td>
<td>1.0047</td>
<td>-0.0348</td>
<td>1</td>
<td>2.422</td>
<td>-</td>
</tr>
<tr>
<td>bc</td>
<td>123.03</td>
<td>-11.29</td>
<td>1</td>
<td>1787.42</td>
<td>-</td>
<td>1.121</td>
<td>-0.0548</td>
<td>1</td>
<td>6.017</td>
<td>1.942</td>
</tr>
<tr>
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<td>56.24</td>
<td>2.31</td>
<td>2</td>
<td>254.71</td>
<td>-</td>
<td>0.8641</td>
<td>-0.0434</td>
<td>1</td>
<td>3.76</td>
<td>-</td>
</tr>
</tbody>
</table>

$\tau$: time at which 63.2 percent of the medicament dissolved, $\beta$: shape parameter.
Microcapsules fraction size: 355-500 μm.
EMS: error mean square.
Significance level based on 1 d.f.; * (p < 0.05), ** (p < 0.01).

*a* See Table 3 for treatment combination key.
Y₂ = 0.3907 + 0.0150X₁ + 0.01141X₂ +
     0.0549X₁X₂ - 0.00214X₁X₃ - 0.0149X₁X₄ -
     0.0186X₁X₅ - 0.000413X₂X₃ . . . . . . . . . (5)

Y₃ = 25.41 - 2.374X₁ + 5.779X₂ + 0.4263X₃ -
     0.714X₁X₂ - 0.0563X₁X₃ - 0.509X₁X₄ -
     0.1513X₁X₂X₃ . . . . . . . . . . . . . . . . . (6)

The responses measured on the micromeritic properties were: Y₁, the geometric mean particle
 diameter (dg) value; Y₂, span value (S) and Y₃, percentage of drug content value. The coded
 independent formulation variables X₁, X₂ and X₃ represent the variables A (polymers blend
 concentration), B (amount of allopurinol) and C (type of coacervation-inducing agent),
 respectively.

The positive sign of the regression coefficients refers to an increasing effect while the
 negative sign indicates a decreasing effect on the corresponding response. Thus, according to
 effectiveness coefficients, the sequence of factors effectiveness was found to be in the following
 orders:

For the geometric mean particle diameter (dg): type of coacervation-inducing agent (X₃) >
 polymers blend concentration (X₁) > amount of allopurinol (X₂). It can be easily noted from
 the polynomial equation No. 4 that the value of dg decreases with rising factor level, i.e., with
 using PIB as a coacervation-inducing agent. The ANOVA results revealed that dg was mainly
 affected by factor X₁ at the 95 percent probability level (Table 4).

For the span value (S): X₃ > X₁ > X₂. The polynomial equation No. 5 indicates that the
 value of (S) rises with rising factor value i.e., with employing PIB and higher polymers blend
 concentration in the phase-separation process. Factors X₁ and X₂ are effective at p < 0.05,
 whereas, factor X₃ (the most effective one) has better significance (p < 0.01). Interaction of
 the factors (X₁X₂ and X₁X₃) was negative and significant (Table 4).

For the percentage of drug content (Y₃): X₂ > X₁ > X₃. The effect of factors X₁ and X₂ on
 the drug content percent has significance (p < 0.05), whereas factor X₃ is non-effective (Table
 4). As exhibited from the polynomial equation

No. 6, the drug content increases with increasing
amount of allopurinol (X₂) and with decreasing
the polymers blend concentration (factor X₁).

Evaluation of the factorial design release
results of allopurinol-loaded polyvinyl chloride/
poloxamer 188 microcapsules

In order to indicate the influence of the selected factors (polymers blend concentration
(A), amount of allopurinol (B), and type of coacervation-inducing agent (C)) and their
interactions in the factorial design of experiments on the dissolution kinetic, the
parameters: (τ: the time at which 63.2 percent of the medicament dissolved and β: the shape
parameter) of the RRSBW distribution were selected. In most of cases, the highest
determination coefficients and the best linear relation (0.5-4 h) were observed form polyvinyl
chloride/poloxamer 188 microcapsules by the
RRSBW distribution.

The results obtained revealed that the τ
values of smaller microcapsules having an
average size of 302.5 μm (data not shown) were
not greatly different from those of bigger
microcapsules of an average size of 427.5 μm
(τ = 2.964 h - 5.151 h, S.I.F. (pH 7.4) in Table
5) when EVA was used as a coacervation-
inducing agent. On the other hand, the estimated
τ values (S.I.F) of the smaller microcapsules
(average size of 302.5 μm, data not shown) were
significantly higher by about 20.56 to 51.02
percent (depending on the preparation
conditions) than those of microcapsules of an
average size of 427.5 μm when PIB was used as a
coacervation-inducing agent.

As can clearly be seen from the RRSBW
distribution plots (Fig. 5), the release profiles in
simulated intestinal fluid (S.I.F, pH 7.4) were
faster than in simulated gastric fluid (S.G.F., pH
1.2).

From the results of the 2³ factorial design
experiments (Table 5), the following polynomials can be derived for the values of τ
and β:

Y₂ = 271.05 - 44.86X₁ + 1.2662X₂ - 53.52X₃ +
     1.054X₁X₂ + 2.211X₁X₃ - 21.27X₁X₄ -
     30.04X₁X₂X₃ . . . . . . . . . . . . . . . . . . . (7)
Fig. 5: RRSBW distribution plots of allopurinol-loaded polyvinyl chloride/polyoxamer 188 microcapsules prepared by using EVA copolymer (A) and PIB (B) as coacervation-inducing agents. Microencapsulation conditions: 33.2% w/w dioctylphthalate, 10% w/w polyoxamer 188 and a non-solvent addition rate of 100 drop/min. (see Table 3 for treatment combinations key).
\[ Y^* = 0.9485 - 0.01885X_1 + 0.02805X_2 + 0.0349X_1 + 0.01405X_2X_2 + 0.03718X_1X_2 + 0.0691X_1X_3 + 0.00513X_1X_3 \ldots \] (8)

\[ Y^*_s = 176.89 - 32.35X_1 - 10.355X_2 - 71.255X_3 - 17.153X_1X_2 + 14.95X_1X_3 - 5.643X_2X_3 + 1.155X_1X_2X_3 \ldots \] (9)

\[ Y^*_s = 1.0256 - 0.06965X_1 - 0.001475X_2 - 0.00415X_1 - 0.01973X_2 - 0.0174X_1X_3 - 0.0327X_1X_2 - 0.021675X_1X_3 \ldots \] (10)

The responses measured on the resulting dissolution parameters of allopurinol microcapsules were: \( Y_o, Y_s \) for \( \tau \) values of the release from the microcapsules of an average size of 427.5 \( \mu \)m in S.G.F. (pH 1.2) and S.I.F. (pH 7.4), respectively and \( Y^*_o, Y^*_s \) for \( \beta \) values in the same sequence of the dissolution media. The coded independent formulation variables \( X_1, X_2 \) and \( X_3 \) represent variables A, B and C, respectively.

In general, the polynomial equations for the effect of the formulation factors on the \( \tau \) values revealed that the effectivity range of the factors was in the following order: type of coacervation-inducing agent \( (X_1) \) > polymers blend concentration \( (X_2) \) > amount of allopurinol \( (X_3) \). It is clearly indicated from the polynomial equations (No’s 7 & 9) that the \( \tau \) value increases with decreasing the levels of factors \( X_1 \) and \( X_2 \) as well as with \( X_3 \), especially for the release experiments in S.I.F. (pH 7.4). In most of the cases, the influence of these factors on the \( \tau \) value had significance \( (p < 0.01) \) irrespective of the pH of the dissolution media (Table 5). However, factors \( X_4, X_2 \) and \( X_3 \) were also negative and significant \( (p < 0.01) \) when smaller microcapsules of an average size of 302.5 \( \mu \)m were utilized in the dissolution experiments in S.I.F. (data not shown). The \( X_1X_4 \) interactions were positive and effective for microcapsules having average sizes of 427.5 \( \mu \)m (Table 5) and 302.5 \( \mu \)m \( (p < 0.05) \), when tested in S.I.F. (pH 7.4), whereas interactions of the factors \( X_2X_3 \) and \( X_1X_2X_3 \) were negative and significant \( (p < 0.01, \text{S.G.F. pH 1.2}) \) (Table 5).

With regard to the \( \beta \) value, the polynomial equation (No. 8) indicated that the effectivity range of the factors for the release in S.G.F (pH 1.2) was \( X_4 > X_2 > X_1 \). In addition, a positive synergistic interaction could be observed between \( X_2 \) and \( X_3 \) \( (p < 0.05) \). In S.I.F (pH 7.4), the effectivity sequence of the factors (Eq. No. 10) was \( X_1 > X_3 > X_2 \). Obviously, the factor \( X_1 \) had a significant effect at \( p < 0.05 \) (Table 5).

**Controlled drug release thermodynamics**

Arrhenius plot of diffusion coefficients against the reciprocal of absolute temperature \( T^{-1} \) \((\ln D_o = \ln D_o^* - \text{Ea/R.} \cdot 1/T)\) was applied to detect the temperature dependency of the release process and composite release mechanisms. In this study, \( \ln K \) \((K= \text{release rate constant})\) was plotted against \( T^{-1} \) in place of \( \ln D_o \) to which it is directly related, the other terms being constant (37).

The results shown in Fig. 6 revealed that linearity without a break was observed for allopurinol release from the microcapsules and that the release rates of microcapsules prepared with PIB (activation energy \( (\text{Ea}) = 16.01 \text{ K.cal./mole} \)) were faster than those of microcapsules prepared by using EVA \( (\text{Ea} = 17 \text{ K.cal./mole}) \) as a coacervation-inducing agent at the corresponding temperatures.

**In-vitro release results of tableted microcapsules**

Tableted microcapsules exhibited good physical properties, the faces being hard and shiny with no trace of powder. When the tablets were used in dissolution tests, they had not disintegrated completely at the end of the experiments. However, their shapes and sizes changed during the course of dissolution.

The release profiles of the tested drug, microcapsules and tableted microcapsules are illustrated in Fig. 7. The results indicated that there was a great reduction in the drug release rate from tableted microcapsules compared with the intact drug or untableted microcapsules. The results also revealed that the drug release rate in S.I.F (pH 7.4) was faster than in S.G.F (pH 1.2).
Fig. 6: Arrhenius plots for the release of allopurinol from polyvinyl chloride / poloxamer 188 microcapsules (355–500 μm) prepared by using different coacervation-inducing agents. Microencapsulation conditions: polymers blend concentration: 5% w/v, 33.2% w/w dioctylphthalate, 10% w/w poloxamer 188, amount of allopurinol: 0.454 gm and a non-solvent addition rate of 100 drop/min. Key: EVA \( (r = 0.9992) \) (○), PIB \( (r = 0.9986) \) (●).

Fig. 7: In vitro release of allopurinol from the tableted microcapsules in simulated gastric fluid (A) and simulated intestinal fluid (B). Key: intact drug (○), microcapsules (△), tableted microcapsules (□). Microcapsules (355–500 μm) were prepared by using polymers blend concentration of 5% w/v, allopurinol amount of 0.454 gm, and EVA copolymer non-solvent addition rate of 100 drop/min. Tablet formulations contained an amount of microcapsules equivalent to 50 mg of the drug.
Surface morphology of microcapsules

The results of the scanning electron microscopy are pictorialized in Figs. 8 and 9. The surface morphology of the microcapsules exhibited a high degree of appearance variations in terms of the various preparation variables and indicated that spherical microcapsules were not obtained for any preparation conditions.

Figure 8 shows the influences of dioctyl phthalate (DOP) as a plasticizer and poloxamer 188 (PLX 188) as an added complementary coating material on the surface characteristics of microcapsules prepared using a polymer concentration of 5% w/v, an initial drug amount of 0.227 gm and EVA as a coacervation-inducing agent. Evidently, the representative unplasticized / unblended microcapsules appeared to have well-formed walls but seemed to be aggregated and irregular in shape. They are typified by the presence of many surface imperfections and some macroscopic pores (Fig. 8A, X35, X1000). In addition, the microcapsules have wrinkled and rougher surfaces that are characterized by the existence of distinct aggregates of smaller microcapsules (spherules) and drug particles embedded in the microcapsule membranes (Fig. 8A, X100, X3500).

Typical scanning electron photomicrographs of plasticized microcapsules (which contain 35.5% w/w DOP) demonstrated that free-flowing microcapsules with a better spherical shape and no agglomeration were obtained (Fig. 8B, X35, X100). The surface of the microcapsules appeared to be less porous and characteristically smooth in contrast to unplasticized microcapsules (Fig. 8A). However, numerous drug particles could clearly be seen on the microcapsules surfaces (Fig. 8B, X1000, X3500).

Figure 8C shows that plasticized / blended microcapsules prepared using PLX 188 at a higher concentration (18% w/w based on total polymers weight) are non-spherical. They had furrowed walls and many spherulitic structures on their surfaces (Fig. 8C, X35, X100). The evidence of crystalline allopurinol on the microcapsule surface and from the interior of the microcapsules were encountered for such microcapsules (Fig. 8C X1000, X3500).

Figure 9 shows the influences of the polymers blend concentration and the type of the coacervation-inducing agents on the surface topography of plasticized / blended microcapsules prepared using 33.2% w/w DOP, 10% w/w PLX 188 (based on total polymers weight) and an initial drug amount of 0.454 gm. Thus, using a lower polymers blend concentration of 5% w/v in the presence of EVA as a coacervation-inducing agent under the adopted conditions produced a remarkable improvement in the integrity and particulate regularity of the plasticized / blended microcapsules containing allopurinol (Fig. 9A). However, the micrographs show that the resulting microcapsules were complete-structured, fairly spherical, non-aggregated (Fig. 9A, X35, X100) and relatively smooth and non-porous in nature with no evidence of any surface imperfections or macroscopic pore formations (Fig. 9A, X1000, X3500).

Increasing the polymers blend concentration to 7.5% w/v (at a fixed PLX 188 concentration of 10% w/w based on total polymers weight) appeared to have a pronounced effect on the surface morphology of microcapsules. Generally, the obtained microcapsules seemed to be irregular in shape and formed by agglomeration of smaller ones (Fig. 9B, X35, X100). They possessed an ill-defined internal structure with a distinct evidence of surface deformity, numerous surface drug crystals and macroporous structure. The created bigger pores were occupied by the drug before the dissolution test indicating that microcapsules with thin outer walls were produced (Fig. 9B, X1000, X3500).

In the practical microencapsulation process, when PIB was used as a coacervation-inducing agent, irregular and elongated shape microcapsules were manifested in the representative scanning electron micrographs (Fig. 9C, X35, X100). In addition, the majority of the microcapsule surfaces appeared to be covered and perforated deeply by drug crystals indicating that microcapsules with macroporous structures were formed (Fig. 9C, X1000, X3500).
Fig. 8: Representative scanning electron micrographs for the influences of dioctylphthalate and poloxamer 188 on the surface morphology of allopurinol-loaded polyvinyl chloride microcapsules prepared by using EVA copolymer as a coacervation-inducing agent. Microencapsulation conditions: polymer concentration: 5% w/v, amount of allopurinol: 0.227 gm and a non-solvent addition rate of 100 drop/min. Key: 0% w/w dioctylphthalate, 0% w/w poloxamer 188 (A), 35.5% w/w dioctylphthalate (B), 31.13% w/w dioctylphthalate and 18.08% w/w poloxamer 188 (C).
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DISCUSSION

The developed non-solvent addition coacervation-phase separation method for the manufacture of allopurinol-loaded polyvinyl chloride / poloxamer 188 microcapsules employed an aqueous medium, tetrahydrofurfuryl alcohol, in which the wall-forming medium was dissolved and from which it was caused to coacervate (i.e. separating in the form of viscous liquid droplets) by the addition of an incompatible solvent (cyclohexane : hexane at 1:1 v/v ratio). The coacervate droplets tend to deposit on the surface of the drug particles suspended in the polymer solution and are latter set to a film condition by the rigidization step (i.e. the subsequent cooling of the system which causes further polymer to coacervate and deposit on the already formed walls of polymer coacervate). In particular, without the use of coacervation-inducing agents, a viscous coacervate separates suddenly and regular microcapsules were not practically formed, but matricized core particles appeared. It is noteworthy first that the proper selection of the basic experimental conditions seemed to be of a primary importance to obtain fairly spherical microcapsules having high encapsulation efficiencies (85-99%) and sustained-release properties. As a matter of fact, spherically-shaped particles have several advantages: (i) they have better micromeritic properties, (ii) spherical particles have the free flowability and uniform packability required for pharmaceutical processing and (iii) they are more suitable for coating processes since spherical shape can be uniformly coated with a relatively small amount of polymer.

The plasticization of polymers is important as it may enhance the cohesiveness and formation of spherical and smooth-surfaced microcapsules.77

The present study revealed that dioctylphthalate (DOP) as a plasticizer played a dominant role in producing relatively spherical, smooth and non-aggregated polyvinyl chloride microcapsules (Fig. 8B) which were judged to be materially different from those prepared from the corresponding unplasticized formulations. The largest geometric mean particle diameter (650 µm) obtained with microcapsules M2 (which contain 35.5% w/w DOP, Table 1) may be attributed to the formation of soft and flexible coacervate droplets of bigger size which hardened to larger size microcapsules. Reportedly, plasticized polyvinyl chloride membranes are characterized by low tensile strength and high elongation at break as compared with the rigid polymer.37 This can be explained on the basis that plasticizers lower the thermal properties of the polymer as the glass transition and the minimum film-forming temperatures, therefore elevating elongation at break and backbone flexibility of macromolecule chains under applied stress and reducing brittleness and elastic modulus of the polymer.18,37 Moreover, Wang et al.37 reported that plasticizers such as diethylphthalate at a higher concentration level (30-40% w/w based on the weight of dry polymer) may offer a ductile property to the polymer and change the fracture behaviour from a brittle to a ductile pattern. This transition was due to the shift of the polymeric binder from a glassy to a rubbery state. The latter, in turn, results in the formation of soft and tough polymers, accelerating the film formation process, and providing better wetting of polymers on substrate surfaces, thereby eliminating interfacial discontinuities.18,37,38

The poor surface characteristics (such as surface imperfections, macroscopic pores and irregularity in shape) and sustained-release properties of the unplasticized microcapsules (Fig. 8A) can be explained on the basis of the high rigidity and tensile strength of the thermoplastic vinyl chloride polymer, which may result in a change in the rate of polymer crystallization from organic solvents during microencapsulation and in turn, a change in a net chain contraction and conformation of the resulting membranes. However, the presence of distinct spherulitic structures on the microcapsule surface (Fig. 8A, X35, X100) supports an evidence for rapid polymer crystallization which can thereby affects particle geometry and morphology. Similar morphological structures were observed when rapid crystallization of poly (ε-caprolactone) occurred in organic solvent.32

The modification of microcapsules by the
incorporation of dioctylphthalate as a plasticizer and poloxamer 188 as a complementary coating polymer into the polymer matrix provided a useful mean for preparing plasticized / blended microcapsules having advantageous morphological and sustained-release properties. Thus, it would be necessary to study the parameters influencing microcapsules preparation and kinetic evaluation of their release patterns.

The Rosin-Rimmler-Sperling-Bennett-Weillbull (RRSBW) distribution analysis of the dissolution data can play an important function in product development and may lead to more rational quality control. 34 Like Sevqi et al., 31 the RRSBW distribution with its probability parameters: τ (the time at which 63.2 percent of the medicament dissolved) and β (the shape parameter) appeared as an equation with good fit (determination coefficient (r²) = 0.8931 - 0.9994 within a time range of 0.5-4 h) in defining the release from allopurinol-loaded polyvinyl chloride / poloxamer 188 microcapsules (Fig. 5). In the normal case, the τ parameter will be the most informative parameter which is used in quantitative comparison with other data generated in-vitro or in-vivo, whereas, the shape parameter (β) provides a useful qualitative information on the interaction of the dissolution with other simultaneous processes such as diffusion or disintegration. 34 The faster release rate in simulated intestinal fluid (S.I.F. pH 7.4) than in simulated gastric fluid (S.G.F. pH 1.2) (Fig. 5) was evidently due to allopurinol solubility increase by salt formation in dilute solutions of alkali hydroxides.

The application of factorial design experiments to determine rational limits for critical formulation and/or processing variables has appeared to be extremely useful for the preparation of microcapsules. 28-30 Thus, once the preparation of the plasticized / blended microcapsules was accomplished by the developed method, the effects of several preparative variables (polymers blend concentration (X₁), amount of allopurinol (X₂) and the type of the coacervation-inducing agent (X₃)) and their interactions, whatever they are synergistic or antagonistic on the micrometric and dissolution characteristics of the microcapsules were simultaneously determined by three-factor, two-level (2³) factorial design and described by a first-order polynomial model. The effect of the variable or factor corresponds to the change in response caused by varying its level. In the case where the overall effect is higher or lower than the sum of the individual contributions, the factors interact, synergizing or antagonizing their individual influences.

In this investigation, the largest effect obtained that would be discussed in detail later, was measured for the type of the coacervation-inducing agent and polymers blend concentration factor, whereas the level of allopurinol amount was less important. Disregarding, the higher level combinations (X₁X₂X₃) and the practically non-existent one (X₁X₂X₃), it was also apparent to notice that the effect of the interaction (X₁X₂) between the polymers blend concentration factor and the type of the coacervation-inducing agent on lowering the τ values of microcapsules of an average size of 427.5 μm in S.I.F (pH 7.4) was positive indicating that the factors interaction was antagonizing their individual influences. The X₁X₂ interaction was also positive and significant (p > 0.05) with microcapsules of an average size of 302.5 μm in S.I.F (pH 7.4) (data not shown).

The good monodispersity and particulate integrity, sphericity and regularity of the plasticized / blended microcapsules (Fig. 9A) prepared using a lower polymers blend concentration level (5% w/v at a fixed poloxamer 188 (PLX 188) concentration of 10% w/w based on total polymers weight) and ethylene-vinylacetate copolymer (EVA) as a coacervation-inducing agent may be an important indication that the rate of crystallization of polyvinyl chloride is further retarded by the co-inclusion of dioctylphthalate (DOP) as a plasticizer and a lower concentration level of PLX 188 as a complementary coating polymer into the polymers blend matrix. This might provide an additional plasticization to the polymer and limit the macroporosity development. Huatan et al. 32 reported that the addition of syneronic L61 (poloxamer 181) to poly (e-caprolactone) was accompanied by a
marked fall in the glass transition temperature of the polymer, thereby, giving rise to an increase in segmental chain mobility and usually a decrease in the rate of polymer crystallization.

The irregularity in shape and reduction in size of microcapsules coded M4 (dg: 412.10 μm, Table 1) prepared using a higher concentration level of poloxamer 188 (PLX 188: 18.08% w/w) may be explained on the basis that the degree of microcapsule aggregation decreases and the irregularity prevails with increasing PLX 188 content in the polymers blend. A result that might be due to the rigid property of PLX 188 and its surface active properties which resulted in better mixing of solvent and non-solvent phases and rapid microcapsule formation. The results obtained are consistent with those of Wu et al. who revealed that the smaller size of microcapsule was easily obtained with the higher diffusion rate of a non-solvent to the polymer solution (solvent phase).

Two types of coacervation-inducing agents namely, ethylene-vinylacetate copolymer (EVA) and polyisobutylene (PIB) which appeared to have a profound effect on the successfulness of the microencapsulation process were compared in this report. The mechanism by which the aggregation of encapsulated particles was prevented in presence of a non-wall forming polymer such as PIB could be explained on the basis that the solution of PIB leads to the production of a thin-wall that spreads over the surface of particles, which in the absence of the protective polymer PIB, the wall gets thicker and insufficiently coats the total surface of the particles.

Clearly, the production of more than 5-11% and 46-65% of PIB-microcapsules having average diameters of 200 and 302.5 μm, respectively may be an indication for the formation of smaller microcapsules induced by PIB as a coacervation-inducing agent than with EVA (~5% and 12-60%) of the microcapsules having average diameters of 200 and 302.5 μm, respectively). This might be due to the fact that the viscosity of PIB (MW: 380,000) solutions used was higher than those of EVA. Similar results were obtained by Lin, who studied the influence of coacervation-inducing agents (PIB, EVA) on the preparation of bleomycin hydrochloride microcapsules by temperature-change method and found that higher viscosity induced by PIB could prevent aggregation of microcapsules leading to smaller particle size microcapsules than when EVA was used. Moreover, in the non-solvent addition process, PIB with higher molecular weight (~380,000) produces larger volume of smaller coacervate droplets even at low concentrations and imparts steric stabilization to coacervate droplets due to chain length and interfacial viscosity effects on droplets repulsion. On the other hand, the inefficiency of PIB in producing spherical and monodisperse microcapsules might be ascribed to the observed high viscosity of the continuous phase, so the polymer precipitated irregularly. In contrast, the higher monodispersity (more than 70-80%) of microcapsules prepared using various preparative conditions had an average size of 427.5 μm) and sphericity of EVA-microcapsules might be due to EVA at the appropriate concentration played two roles in the coacervation of polyvinyl chloride. First, it worked as a coacervation-inducing agent. This function was based on the evidence that the coacervation of polyvinyl chloride was generated by smaller amounts of non-solvent (50 ml) poured into the system with EVA than with PIB (volume of non-solvent: 75 ml). Second, it worked as a protective colloid effectively to induce coacervation and to prevent coacervates from aggregation. From the preliminary experimental results, it was observed that the addition of tetrahydrofurfuryl alcohol (THFFA) to the solution of EVA in cyclohexane : hexane (1:1 v/v ratio) resulted in a phase-separation and appearance of a distinct polymer-rich phase. In other words, it could be postulated that EVA-copolymer might be precipitated as a thin film-coat on the surface of coacervate droplets by means of THFFA diffused into the continuous phase during the coacervation process, thereby protecting coacervate droplets against deformation. Thus, the resultant coacervate droplets induced by EVA at the appropriate polymers blend concentration (5% w/v at a fixed PLX 188 concentration of 10% w/w) deposited preferentially on the surface of allopurinol, satisfactorily producing spherical microcapsules of complete and compact outer walls, which
were devoid of surface spherules, surface drug crystals and macroporosity even at a higher initial drug amount. A result which might account for the slower RRSBW release behaviour and the longer \( \tau \) values (the time at which 63.2 percent of the medicament dissolved, \( \tau \) (S.G.F, pH 1.2) = 5.40-6.34 h, \( \tau \) (S.I.F, pH 7.4) = 2.96-5.15 h depending on the preparation conditions) obtained by using EVA as a coacervation-inducing agent in place of PIB (\( \tau \) (S.G.F, pH 1.2) = 2.098-4.49 h, \( \tau \) (S.I.F, pH 7.4) = 0.9373-2.051 h depending on the preparation conditions). Lin and Yang\(^6\) studied the role of EVA copolymer as a coacervation-inducing agent on the preparation and release kinetics of theophylline-ethylcellulose microcapsules and reported that, the higher the concentration of EVA copolymer used in the phase-separation process by temperature-change method, the slower the release rate of the theophylline microcapsules. An effect which might be due to the higher concentration of EVA was more completely inducing ethylcellulose deposition on the drug particles, leading to a thicker wall.

With respect to the polymers blend concentration parameter, the study signified clearly that the release behaviour was related to the degree of coating i.e., the higher the amount of the wall material (polymers blend concentration of 7.5% w/v) in relation to that of the drug, the greater the tendency of a pore formation and the lower the encapsulation efficiencies (85.36-90.33%) which consequently resulted in faster release kinetics, especially when PIB was used as a coacervation-inducing agent (\( \tau \) (S.I.F, pH 7.4) = 0.9373-2.004 h depending on the preparation conditions). In fact, with thicker coating (i.e., higher polymer viscosity), fewer drug particles are expected to be coated resulting in part of the polymer not being taken by the core surface, thereby separating as empty coacervate shells. Similar results were documented by Ndese and \textit{et al.} on the microencapsulation of chloroquine diphosphate by a non-solvent addition phase-separation process.

With respect to the drug release thermodynamics, the temperature dependency (i.e., the higher the temperature, the greater the drug release rate) of allopurinol release from polyvinyl chloride / poloxamer 188 microcapsules indicates clearly that the release process is in accordance with a single mechanism of release and is an energy-linked process. The higher value of the activation energy (\( E_a = 17.0 \text{ Kcal./mole} \)) obtained when using EVA as a coacervation-inducing agent than when PIB (\( E_a = 16.0 \text{ Kcal./mole} \)) was used could possibly be attributed to the porous nature and pronounced permeability of microcapsules prepared with PIB as a coacervation-inducing agent, so a relatively less energy was needed for the drug diffusion process.

Compression of microcapsules into tablets may result either in damage to the microcapsules, leading to increased dissolution rates of the drug from tableted microcapsules, or to delay in the dissolution rates from the non-disintegrating polymer matrix tablet prepared in tableting.\(^4\) However the slower release rates of tableted allopurinol-loaded polyvinyl chloride / poloxamer 188 microcapsules having a zero-order release kinetics (Fig. 7) in comparison with untablled microcapsules could be explained on the basis of the reduced surface area and porosity (channel permeation) of the compressed tablets as well as the improved properties of the tableted microcapsules prepared with EVA as a coacervation-inducing agent. Conversely, the untablled microcapsules were homogenously dispersed in the dissolution medium leading to a relatively rapid release rate, but it did not negate the remarkable sustained-release properties of the prepared microcapsules.

**Conclusion**

The developed non-solvent addition phase-separation technique which employed tetrahydrofurfuryl alcohol-cyclohexane : hexane (1:1 v/v ratio) as a solvent-non solvent pair, a novel binary polymers blend comprising of polyvinyl chloride and poloxamer 188 as the wall materials, dioctylphthalate as a plasticizer and ethylene-vinylacate and polyisobutylene as coacervation-inducing agents proved to be very

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*Ibrahim El-Gibaly*
reliable, reproducible, versatile and shows a considerable potential for efficiently encapsulating allopurinol.

From the results of the 2^k factorial design experiments, it was possible to reach unambiguous conclusion about factor effectiveness that appeared to be in the following order: type of coacervation-inducing agent > polymers blend concentration > amount of allopurinol. Thus, faster release profiles were obtained from microcapsules prepared by using higher polymers blend concentration level and/or polyisobutylene as a coacervation-inducing agent.

The analysis of the micromeritic results of microcapsules indicated that the most effective factor on the geometric mean particle diameter and the span value was the type of the coacervation-inducing agent, whereas the drug entrapment ratio was affected mainly by the drug loading factor.

In general, the surface morphology of the microcapsules was closely related to their dissolution rates and affected significantly by the utilization of dioctylphthalate as a plasticizer and poloxamer 188 as a complementary coating polymer in the microcapsules preparation.

The release profiles of the microcapsules fitted the RRSBW distribution model (r^2 = 0.8923-0.9994) with β (shape factor) values in the range of 0.8 to approximately one.

Of special concern, the optimal conditions proposed by means of the factorial design experiments for manufacturing fairly spherical plasticized / blended microcapsules having dense structure, high monodispersity (span value = 0.3374, more than 70% of microcapsules were ranging in size between 355 and 500 µm in diameter) and desired sustained-release properties (τ, the time at which 63.2 percent of the medicament dissolved = 6.05 h (S.G.F., pH 1.2) - 5.15 h (S.I.F., pH 7.4) were obtained for the treatment combination (b) i.e.; with a total polymers blend concentration of 5% w/v at a fixed poloxamer 188 concentration of 10% w/w (based on the dry weight of polymers), allopurinol amount of 0.454 gm and ethylene-vinylacetate copolymer as a coacervation-inducing agent.

In particular, the suggested EVA-microcapsules containing allopurinol with their good physical and sustained-release characteristics can be filled into gelatin capsules as a prolonged-release dosage form for allopurinol. Moreover they appeared to be suitable for further formulation into tablets which, in turn, reduced greatly the drug release rate.

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