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Effect of hexacosanol on the characteristics of novel sustained-release allopurinol solid lipospheres (SLS): factorial design application and product evaluation

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Abstract

This investigation involved the evaluation of the effect of hexacosanol (HC, ceryl alcohol), a new hydrophobic wax modifier (WM) in comparison with conventional modifiers, on the development of sustained-release allopurinol (AP) solid lipospheres (SLS) intended for use in a suspension formulation and other oral dosage forms. Various beeswax (BW)/WM blends (composition ratio 1:1) were thus used to prepare SLS by a modified oil-in-water emulsion meltable disperse-phase (MDP) encapsulation method without using organic solvents and the influence of these blends on the drug encapsulation efficiency (EE), size distribution and the time for 50% of the drug to be released ($t_{50\%}$) was investigated. Results indicated that incorporation of HC in wall matrix of SLS provided the means to enhance the EE of AP and to modulate the rate of drug release into dissolution media (simulated gastric fluid (S.G.F.: pH 1.2) and simulated intestinal fluid (S.I.F.: pH 7.4). The effects of the process variables; HC concentration, dispersant (pluronic F-68: PF-68) concentration and drug:wax ratio were also studied on the properties of AP-loaded SLS by a 2^3 factorial design. The EE values were in the range of 80.8–92.67%. The only significant parameter affecting ($P < 0.01$) the size and size distribution of the SLS formulations was the amount of the PF-68, whereas the factor with the biggest influence ($P < 0.05$) on the drug EE was the initial loading of AP (in terms of the drug:wax ratio). The amount of HC blended with wax and the initial drug loading significantly ($P < 0.01$) affected the $t_{50\%}$ values of all of the formulations. The release of AP was more extended ($t_{50\%}$ values (S.I.F.; pH 7.4) = 9.91–25.36 h, depending on the drug:wax ratio) and surface morphology of SLS was improved with higher HC content (15%, w/w) formulations. The release patterns fitted the Baker–Lonsdale dissolution kinetics for spherical matrices. A significant decrease of plasma uric acid levels ($P < 0.05$) and hepatic impairment in male rats was observed after oral administration of a SLS (mean size: 120 μm) suspensions of the optimum formulation, compared to suspensions of pure AP.

Keywords: Allopurinol; Sustained-release solid lipospheres; Hexacosanol; Melt dispersion; Histopathology

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1. Introduction

Multiple-unit systems offer the advantage that they distribute more uniformly in the gastrointestinal tract (G.I.T.), resulting in a more uniform drug absorption, low possibility of dose dumping and reduced local irritation, when compared to single-unit dosage forms on chronic dosing (Bodmeier et al., 1990). Moreover, they allow the administration of much smaller doses than are normally required because their release is less variable and less dependent on gastric transit time (Demirel et al., 2001).

Various techniques of microencapsulation can be employed for the production of multiparticulate sustained-release polymeric drug delivery systems (Deasy, 1984; Arshady, 1989). However, the toxicity of the organic solvent residues in the final microspheres and the use of potentially toxic monomers are major problems in the conventional microencapsulation process (Arshady, 1989). Therefore, the oil-in-water meltable disperse-phase (MDP) encapsulation method has been reported to be simple and useful in the preparation of beeswax (BW) matrix microspheres loaded with appropriate drugs without using harmful organic solvents (Bodmeier et al., 1992; Adeyeye and Price, 1994; Giannola et al., 1995; Uddin et al., 2001). In fact, microsphere characteristics are greatly affected by processing and formulation variables, such as type of wax, drug loading, type and amount of emulsifier and emulsification stirrer speed (Adeyeye and Price, 1991; Bodmeier et al., 1992; Lee et al., 1998; Varshosaz and Keihanfar, 2001). An increase in drug loading in matrix microspheres usually increases drug release rate (Adeyeye and Price, 1994; Lewis et al., 1998), but may have little effect in certain cases (Varshosaz and Keihanfar, 2001). Benita et al. (1986) reported that the surfactant concentration used during preparation of 5-fluorouracil carnauba wax microspheres by a meltable dispersion method had no effect on particle size distribution, whilst increasing the emulsification speed decreased the mean size of the microspheres. Recently, Varshosaz and Keihanfar (2001) used this method to prepare propranolol wax microspheres and revealed that increasing the surfactant concentration in the dispersing solution or the stirring speed used during the emulsification process decreased the mean particle size of the microspheres.

In the production of microspheres using the melt dispersion technique, various materials (such as cetostearyl alcohol (Cs.A), stearic acid (SAc), stearyl alcohol (St.A), glycerol esters of various fatty acids and polyethylene glycol) have been employed as substrates and/or wax modifiers (WM) to modulate microsphere characteristics (Vilivalam and Adeyeye, 1994; Giannola and De Caro, 1997; Lee et al., 1998). Adeyeye and Price (1994) have documented the importance of incorporating WM (such as St.A or glycerol monostearate (GMS)) in sustained-release ibuprofen-ceresine wax microspheres as an approach to improve their slow dissolution profiles and incomplete release. Also, incorporation of steareth-20 (ethoxylated St.A) in propylene glycol monostearate matrices exerted a strong and unique concentration-dependent effect on size and release characteristics of the resulting microspheres (Treki et al., 1997). Further investigation of the effect of type and amount of WM on properties of the microspheres is still needed for developing sustained-release wax-matrix multiple-unit. Therefore, this current study involves the use of a new hydrophobic WM, hexacosanol (HC) in the formation of wax-based matrices of microspheres as a mean to control their release rates.

Allopurinol (AP), a hyperuricemic agent used mainly to treat gout and leishmaniasis (Martindale, 1999) was selected as a model drug. Its plasma half-life is about 1–3 h. Direct use of AP can have several side effects on the skin (e.g. severe skin rashes) which appear in the form of fevering, shivering, and vasculitis leading to renal and hepatic damage (Martindale, 1999). In addition, adverse effects on the G.I.T. (such as nausea, vomiting and diarrhoea) are common. Relatively limited work has been reported on the microencapsulation of AP (Spiegl and Abd-El-Fattah, 1977), which can reduce any side effects to a great extent. Arabi et al. (1996) produced AP-loaded ethylcellulose microcapsules using the solvent evaporation method and reported the effect of some formulation parameters on the release of the drug from the microcapsules. A fully improved non-solvent addition technique has been developed for encapsulating AP (El-Gibaly, 1998). The optimum microcapsule formulation had adequate drug content and $t_{50\%}$ values (4–5 h). However, these methods require organic solvents, the formed particles were relatively large (>200 μm) and may not be suitable for an oral suspension formulation that requires smaller

particles to avoid gritty sensation during administration (Shah and Chafetz, 1994; Lewis et al., 1998).

The objective of the investigation was to develop a sustained-release SLS of AP using a modified emulsion (MDP) technique, which, due to the gastroresistant wax matrix used and prolonged release properties, may hinder drug discharge in the stomach, minimize unwanted effects of the drug on G.I.T and liver and reduce serum uric acid levels. A 2^3 factorial experiment was designed for this study and the influences of the selected formulation factors (HC concentration, PF-68 concentration in the external aqueous phase and the initial amount of drug being encapsulated) on the characteristics of the formed SLS were determined. Information from SLS studies would be useful for formulation of oral sustained-release suspensions and other oral dosage forms, as well as for further in vivo investigations.

2. Materials and methods

2.1. Materials

Allopurinol (AP) and 1-hexacosanol (HC: $C_{26}H_{54}O$, mol. wt.: 382.7) were obtained from the Sigma Chemical Co. (St. Louis, MO, USA). Bees wax (BW) was donated from Frank B. Ross Company (NJ, USA). Stearyl alcohol (St.A), cetostearyl alcohol (Cs.A), stearic acid (SAc) were brought from Fisher scientific Co. (Fairlawn, NJ). Palmitic acid (PAc) was obtained from BASF (Ludwigshafen, Germany) and pluronic-F68 (PF-68) was supplied by ICI surfactants (Cleveland, UK). All other materials used in this study were of analytical reagent grade and were used as received.

2.2. Methods

2.2.1. Factorial design experiments

Preliminary studies were carried out to identify the process/formulation variables (Table 1). SLS formulations were produced, based on the 2^3 randomized factorial design. The independent variables are HC concentration (X_1), PF-68 concentration in the external aqueous phase (X_2) and drug:wax weight ratio (X_3). The independent variables and their levels are shown in Table 2a. On the other hand, the time for 50% of the drug to be released [$t_{50\%}$ (h), S.I.F. (pH 7.4)] (Y_1), the drug encapsulation efficiency (EE) of the SLS formulations [(EE%) (Y_2)], the geometric mean particle diameter, d_g (μm) (Y_3) and the % yield of SLS of mean size <200 μm (Y_4) are the dependent variables (response parameters). Table 2b summarizes the factorial study experimental design and the values for the independent and dependent variables. Fitting a multiple linear regression model to a 2^3 factorial design gave a predictor equation which was a first-order polynomial, having the form (Cohran and Cox, 1957; Bolton, 1984):

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3$$

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

Statistical analysis of results was performed using analysis of variance (ANOVA) and regression coefficients of all factors and interaction terms were calculated (Bolton, 1984) (Table 3).

2.2.2. Preparation of AP-loaded SLS formulations

SLS formulations are produced using the modified emulsion (MDP) encapsulation procedure adopted (Vilivalam and Adeyeye, 1994). The SLS formulations were prepared by melting the wax components (4 g), unless otherwise specified, consisting of beeswax (BW) and wax modifiers (WM) (St.A and HC) (BW:WM ratio of 1:1). This constitutes the organic or oil phase (Table 1). The drug was dispersed in the respective molten wax (in a 1:4 drug to wax ratio) under continuous stirring using a magnetic stirrer (J.P. Selecta, s.a., Spain) until a homogeneous blend was attained. This mixture was then poured into 100 ml of the encapsulation dispersant (PF-68: 0.25 g) solution (aqueous phase) with constant stirring using a mechanical stirrer (Wheaton Instruments, Millville, NJ, USA) at a predetermined speed of 400 rpm to form an oil-in-water emulsion. During the emulsification step (1 min), the temperature of the aqueous phase was kept slightly higher (∼5 °C) than the highest melting point of any of the components of the oil phase. The molten mass upon dispersion in the aqueous medium formed spherical oily particles, which solidified enveloping the drug upon rapid cooling of the emulsion using chilled water (4 °C). The resultant SLS particles were then filtered extensively washed with deionized

Table 1
Preliminary AP-loaded SLS formulations using a 1:4 (drug:wax) ratio

Variables	Values	Code	Drug (g)	Wax (g)	Wax modifier[a] (g)	Actual drug content (%, w/w ± S.D.)	Drug EE%[b] (w/w)	$t_{50\%}$ (S.I.F.)[b] (h ± S.D.)[c]	d_g (μm)	σ_g
Type of wax modifier	–	A	1	2	Ct.A (2)	11.78 ± 1.714	58.92	<0.25 (0.25)	725	1.48
	–	B	1	2	St.A (2)	13.60 ± 1.061	68.01	1.04 ± 0.0495 (12.5)	646	1.41
	–	C	1	2	Cs.A (2)	13.04 ± 1.020	65.20	0.385 ± 0.022 (2.5)	519	1.28
	–	D	1	2	HC (2)	18.40 ± 0.296	92.01	−e (−)[d]	741	1.45
	–	E	1	2	PAc (2)	9.67 ± 0.728	48.35	<0.25 (≤0.25)	708	1.57
	–	F	1	2	SAc (2)	9.30 ± 0.051	46.48	<0.25 (<0.25)	989	1.53
Wax:St.A ratio	1.5:1	G	1	2.4	St.A (1.6)	13.28 ± 0.2772	66.42	0.614 ± 0.013 (5)	596	1.40
	2:1	H	1	2.67	St.A (1.33)	13.31 ± 0.0370	66.56	<0.25 (1.01)	759	1.40
HC concentration (%, w/w)[e]	2.5	I	1	2	St.A (1.9) HC (0.1)	16.26 ± 0.6251	81.31	2.02 ± 0.092 (−)	576	1.31
	5	J	1	2	St.A (1.8) HC (0.2)	16.07 ± 1.363	80.35	2.34 ± 0.432 (−)	442	1.30
	10	K	1	2	St.A (1.6) HC (0.4)	16.16 ± 0.396	80.80	5.89 ± 0.506 (−)	550	1.41

[a] Ct.A: cetyl alcohol, St.A: stearyl alcohol, Cs.A: cetostearyl alcohol, HC: hexacosanol, PAc: palmitic acid, SAc: stearic acid.
[b] Drug EE% and $t_{50\%}$ values (h) were calculated from the 550 μm particles.
[c] Figures in parentheses indicate release in S.G.F. (pH 1.2).
[d] SLS released <3% of their payload after 4 and 12 h of dissolution testing in S.G.F. and S.I.F., respectively.
[e] Defined as weight of HC per total weight of waxes.

Table 2
A 2^3 factorial design parameters and experimental conditions of SLS formulations

Factors	Low level (−)	High level (+)
(a) The independent variables and their levels		
(A) HC concentration (%, w/w)[a]	10	15
(B) Concentration of PF-68 (%, w/v)	0.25	0.75
(C) Drug:wax ratio	1:4	1:2.29

(b) Formulation of the SLS utilizing 2^3 factorial design

Code	X_1	X_2	X_3	Y_1[b]	Y_2[b]	Y_3	Y_4
L1	10	0.25	1:4	5.89 ± 0.506	80.80 ± 3.89 (16.16)[c]	550 (1.41)[d]	5.980
L2	15	0.25	1:4	25.36 ± 3.46	86.30 ± 4.35 (17.26)	589 (1.50)	7.411
L3	10	0.75	1:4	6.98 ± 0.917	88.32 ± 4.98 (17.66)	324 (1.34)	37.25
L4	15	0.75	1:4	20.30 ± 3.80	82.40 ± 0.980 (16.48)	447 (1.26)	14.73
L5	10	0.25	1:2.29	3.12 ± 0.340	81.63 ± 0.311 (24.84)	531 (1.34)	6.120
L6	15	0.25	1:2.29	9.91 ± 0.260	89.85 ± 3.370 (27.34)	442 (1.38)	10.66
L7	10	0.75	1:2.29	5.68 ± 0.276	92.67 ± 0.770 (28.20)	447 (1.29)	12.09
L8	15	0.75	1:2.29	11.89 ± 0.351	90.18 ± 2.33 (27.44)	469 (1.39)	14.84

The independent variables are HC concentration (X_1), PF-68 concentration in the external aqueous phase (X_2) and the drug:wax ratio (X_3). Drug release ($t_{50\%}$ (h), S.I.F. (pH 7.4)) (Y_1); drug EE% (Y_2), d_g (μm) (Y_3) and % yield of SLS of mean size <200 μm (Y_4) are the dependent variables. Drug EE% and $t_{50\%}$ values (h) were calculated from the 550 μm particles.
[a] Defined as weight of HC per total weight of waxes.
[b] Mean values ± S.D. ($n = 3$).
[c] Data between parentheses indicate the actual drug content (mean values (%, w/w), $n = 6$).
[d] Data between parentheses indicate σ_g.

Table 3
Results of analysis of variance for 2^3 factorial experiments of SLS formulations[a] (run in triplicate)

Code	Source of variation[b]	Experiment 1	Experiment 2	Experiment 3	d.f.	Mean square	*F* ratio
L1	(1)	5.91	6.40	5.40			
L2	(a)	21.4	26.85	27.83	1	87.291	25.0082**
L3	(b)	8.01	6.23	6.70	1	0.01293	0.003704
L4	(ab)	16.5	24.1	20.3	1	1.8833	0.53955
L5	(c)	3.01	2.85	3.50	1	32.534	9.3207**
L6	(ac)	10.2	9.70	9.83	1	16.307	4.6718*
L7	(bc)	6.01	5.49	5.56	1	3.0267	0.86713
L8	(abc)	11.55	11.86	12.25	1	1.284	0.36786
	Experimental error				16	3.4905	
	Significance level based on d.f.				1		

The effect of independent variables (HC concentration (a), PF-68 concentration in the external aqueous phase (b) and the drug:wax ratio (c)) on the $t_{50\%}$ values (h, S.I.F.: pH 7.4) (Y_1).

[a] Mean size: 550 μm.

[b] (1) Refers to all factors at their low levels; if factor A is at its high level and B and C are at their low levels, the combination is denoted as (a), etc.

* $P < 0.05$.

** $P < 0.01$.

water to remove any surfactant or drug residues, and air-dried at room temperature for 48 h. Blank SLS formulations (without drug) were similarly prepared. All batches were prepared in triplicate.

Different blends and ratios of BW and WM were used in the preliminary SLS formulations using a 1:4 (drug:wax) ratio (Table 1) to select the best blends (to obtain discrete, essentially non-aggregate and free-flowing SLS with high yields and drug content) for further studies. A number of different process/formulation variables were thus investigated for optimization of a SLS formulation properties prepared by using 2^3 factorial design experiments (Tables 2 and 3).

The influence of the emulsification stirrer speed at elevated temperature prior to cooling on the characteristics of the resulting SLS formulations was also examined using two different speeds (400 and 600 rpm).

2.2.3. Microscopic evaluation of AP-loaded SLS formulations

The detailed surface characteristics of the formulated SLS were observed using a scanning electron microscope (JEOL JSM-5200 SEM). The samples were gold-coated under vacuum using a sputter coater (JFC-1100E ion sputtering device, JEOL) and then investigated at 15 kV. The morphology of the optimum drug-loaded SLS formulation (code L2; mean size: 550 μm) (Table 2) at 0 h (before dissolution) and after 24 h dissolution using enzyme-free simulated intestinal fluid (S.I.F.: KH_2PO_4/NaOH buffer solution (pH 7.4)) was also studied.

2.2.4. X-ray diffraction studies

X-ray diffraction analysis was performed on code L2 formulation with 17.26 ± 1.393% (w/w) drug loading (Table 2) to determine the crystalline state of the drug within the particles using a Phillips X-ray powder diffractometer (Phillips PW-1710 generator, The Netherlands) with a Ni-filtered Cu Kα-radiation and a scanning speed of 5 °C/min.

2.2.5. Encapsulation efficiency (EE)

To determine the EE of AP, the SLS formulations were assayed for drug content. Fifty milligrams of SLS formulation of each batch were crushed in a clean mortar and pulverized by the aid of a small amount of enzyme-free S.I.F (KH_2PO_4/NaOH buffer, pH 7.4), then digested in 100 ml of the same media and extracted completely during a period of 12 h. The solution was filtered and the amount of AP was measured spectrophotometrically (Shimadzu, Double-Beam Spectrophotometer 150-02, Japan) at 250 nm against a blank prepared using blank SLS formulations. The maximum extraction of AP from the SLS formulations was considered to have been achieved when a constant concentration of drug was measured. Preliminary results indicated that wax materials did not influence complete core extraction or interfere with the

analytical method. The percentage drug content (determined in triplicate for each batch) and drug EE(%) were calculated using the following equations:

$$\text{drug content (\%)} = \frac{\text{amount of drug in SLS}}{\text{amount of SLS}} \times 100$$

$$\text{EE (\%)} = \frac{\text{\% drug content}}{\text{\% theoretical content}} \times 100$$

2.2.6. In vitro release characteristics

The in vitro dissolution test of AP from SLS formulations (equivalent to 20 mg of drug) was performed in triplicate using the USP dissolution rotating basket assembly (Model DT-06, Erweka, Germany) at a stirring speed of 50 ± 1 rpm and a temperature of $37 \pm 0.5\,^{\circ}$C. Five hundred milliliters of simulated gastric fluid (S.G.F.: HCl/NaCl buffer solution, pH 1.2) and (S.I.F.: KH_2PO_4/NaOH buffer solution, pH 7.4), without enzymes but with 0.02%. Tween 80, were used as dissolution media. The dissolution samples (5 ml) were withdrawn at specified time intervals, replaced with equal volumes of preheated dissolution media and analysed spectrophotometrically at 250 nm. The UV-absorption with blank SLS formulations in dissolution test conditions was also measured.

The formulation and optimization parameters investigated in the dissolution studies were WM type, BW:St.A ratio, HC and PF-68 concentrations; drug loading and SLS size (Tables 1–3).

2.2.7. Kinetic analysis of release data

The release mechanism of AP from SLS formulations was investigated using data corresponding to the first 60% of drug released according to the following mathematical models (Table 4).

2.2.7.1. Ritger–Peppas model. This model is often applied to describe drug release from matrix systems of various geometries by using a simple exponential relation (Ritger and Peppas, 1987):

$$\frac{M_t}{M_\infty} = Kt^n$$

where M_t/M_∞ denotes the fraction of drug released at time t, K the release rate constant characteristic of the controlled-release device and n the diffusional

Table 4
Kinetic assessment of dissolution data for L1–L8 SLS formulations[a] (S.I.F., pH 7.4)

Kinetic model	Regression parameters[b]	L1	L2	L3	L4	L5	L6	L7	L8
Ritger–Peppas	r^2	0.9876 (0.0058)	0.9773 (0.0031)	0.9856 (0.0026)	0.9546 (0.0094)	0.9789 (0.0207)	0.9663 (0.0021)	0.9845 (0.0019)	0.9550 (0.0076)
	n	0.3494 (0.0243)	0.3142 (0.0547)	0.3620 (0.0460)	0.2640 (0.0216)	0.3280 (0.0094)	0.2520 (0.0202)	0.4326 (0.0355)	0.290 (0.0167)
	Intercept	1.062 (0.0451)	0.8391 (0.1057)	1.014 (0.0856)	1.028 (0.0376)	1.205 (0.1106)	1.178 (0.0195)	0.9412 (0.0553)	1.058 (0.0262)
Jander's equation	r^2	0.9908 (0.0013)	0.9923 (0.00071)	0.9876 (0.0129)	0.9846 (0.0048)	0.9906 (0.0036)	0.9825 (0.0031)	0.9829 (0.0026)	0.9686 (0.0068)
	$K_J \times 10^{-2}$ ($h^{-1/2}$)	7.864 (0.0109)	3.020 (0.0044)	7.473 (0.0135)	3.552 (0.0039)	11.12 (0.00398)	4.988 (0.00689)	9.794 (0.01092)	4.850 (0.00296)
	Intercept	0.0181 (0.0097)	0.0189 (0.0098)	0.0110 (0.00078)	0.0334 (0.0055)	0.0214 (0.0086)	0.0466 (0.0063)	0.0167 (0.0021)	0.0287 (0.0043)
Baker–Lonsdale	r^2	0.9977 (0.0017)	0.9981 (0.0098)	0.9951 (0.0029)	0.9977 (0.0103)	0.9978 (0.0033)	0.9952 (0.0048)	0.9928 (0.0051)	0.9847 (0.0098)
	$K_{BL} \times 10^{-3}$ (h^{-1})	9.009 (0.00186)	1.665 (0.00028)	7.825 (0.00113)	2.525 (0.00044)	16.11 (0.00068)	4.718 (0.00089)	11.15 (0.00140)	4.160 (0.00032)
	Intercept	0.00382 (0.0016)	0.00184 (0.0013)	0.00211 (0.0023)	0.00427 (0.00082)	0.00788 (0.0021)	0.00832 (0.00093)	0.00294 (0.0022)	0.0039 (0.00049)

Regression parameters (r^2: determination coefficients, n: diffusional release exponents, K_J: Jander's rate constant, K_{BL}: Baker–Lonsdale model rate constant) were calculated using data for the time interval from 0.5 to 12 h.

[a] Mean size: 550 μm.

[b] Figures in parentheses indicate S.D. ($n = 3$).

exponent, indicative of the mechanism of drug release. In spherical matrices, if $n \leq 0.43$, a Fickian diffusion (case-I), $0.43 \leq n < 0.85$, a non-Fickian transport and $n \geq 0.85$, a case-II transport (zero order) drug release mechanism dominates (Ritger and Peppas, 1987; Varshosaz and Keihanfar, 2001).

2.2.7.2. Jander's equation model. This model considers the change in the interfacial area where the actual release of a solid drug from microspheres (which do not change in shape during drug release) occurs by diffusion within the micromatrix (Jander, 1927; Akiyama et al., 1993). In this case, the drug release kinetics may be expressed as follows:

$$1 - \left(1 - \frac{M_t}{M_\infty}\right)^{1/3} = \left(\frac{K_1}{r}\right) t^{1/2} = K_J t^{1/2}$$

where K_1 and K_J are the rate constants and r the radius of the spherical matrix.

2.2.7.3. Baker–Lonsdale model. For a system consisting of drugs dispersed homogeneously or heterogeneously (i.e. drug is insoluble in the matrix system) within a spherical diffusion rate-limiting matrix, the drug release can be satisfactorily modelled to the following equation (Baker and Lonsdale, 1974):

$$\frac{3}{2}\left[1 - \left(1 - \frac{M_t}{M_\infty}\right)^{2/3}\right] - \frac{M_t}{M_\infty} = \left(\frac{K_2}{r^2}\right) t = K_{BL} t$$

where K_2 and K_{BL} are the rate constants and r the radius of the spherical matrix.

2.2.8. In vivo studies

In order to compare the plasma uric acid levels and hepatic changes of pure AP with AP-loaded SLS formulations. The optimum SLS formulation (code L2, mean size: 120 μm) was tested in rats. Thirty healthy male rats, weighing 150–200 g, were kept on a standard diet throughout the experimentation period. Animals were divided into three groups (10 rats each) and received the tested compounds by gastric intubation (in 0.1 M $NaHCO_3$ containing 0.02% of Tween 80, pH 7.4) daily for 3 months. Group one (the control) received vehicle only. Group two were given AP suspension (5 mg/ml AP) at a dose of 25 mg/kg of body weight. Group three received SLS formulation (code L2 (mean size: 120 μm) equivalent to 25 mg of AP per kg of body weight). All treatments were administered into rats following a minimum of 12 h of fasting. At the end of the test period, blood samples were taken from the rats using heparinized injectors, and plasmas were separated by centrifugation at 3000 rpm for 5 min. Plasma samples were kept at −20 °C until analysis of uric acid concentration. Livers were excised immediately after rats scarification. Specimens of liver were taken for histopathological examination.

2.2.9. Determination of uric acid level

Uric acid was measured in the plasma of rats colorimetrically after deproteinization by phosphotungstic reduction in an alkaline medium (Caraway, 1963) using a uric acid-kit (BioMe'rieux laboratory reagents and products, France).

2.2.10. Histopathological examination

Some specimens from livers were fixed in 10% neutral buffer formalin, then dehydrated, cleaned and embedded in paraffin. Sections of average 5 μm in thickness were taken and stained with haematoxylin (H) and eosin (E) stains according to Bancroft and Stevens (1982), then examined by light microscopy.

3. Results and discussion

3.1. Preparation and evaluation of AP-loaded SLS formulations

The technique used in this study, involving cooling-induced solidification of the meltable oily phase of a modified two-phase system, is advantageous for the entrapment of water insoluble drugs (Bodmeier et al., 1992). The white BW due to its physical properties and behaviour in the intestinal lumen (Giannola et al., 1995) was used to prepare gastroresistant SLS formulations using the adopted technique. However, when BW alone was used as the oil phase, the droplets rapidly coalesced and formed big lumps upon cooling. Therefore, to modify the properties of BW-based SLS, various WM of low-viscosity melts (cetyl alcohol (Ct.A), stearyl alcohol (St.A), cetostearyl alcohol (Cs.A), hexacosanol (HC), palmitic acid (PAc) and stearic acid (SAc)) were selected for the formulations, based on preliminary studies. The dispersant (PF-68) was used at a minimal concentration (0.25%, w/v) to stabilize

the oil-in-water emulsions by reducing the high interfacial tension between the hydrophobic material and the aqueous external phase producing wettable oleaginous material and individual microparticles upon cooling.

The preliminary SLS formulations consisting of blends of BW and WM at 1:1 ratio, where a 1:4 (drug:wax) ratio was used, are given in Table 1. The production yield of SLS prepared from all formulations were high (>90%). The slight loss of solids was due to congealed matrix on the glass wall during solidification. The formulated SLS followed a log-normal distribution, the geometric mean particle diameter, d_g (μm) and geometric standard deviation, σ_g parameters were calculated for the formulations as shown in Table 1. Incorporation of WM significantly affected the size distribution of the formed SLS (codes A–F) which had a d_g in the particle size range of 519–989 μm and σ_g in the range of 1.28–1.57. The SLS of the formulations B and C, corresponding to BW:St.A (or Cs.A) blends had the smallest d_g (646 and 519 μm, respectively) amongst the tested blends.

The average drug loadings, the drug EE and $t_{50\%}$ values of the 550 μm SLS prepared with different modifiers (in BW/WM ratios of 1:1) are reported in Table 1. Dry SLS (codes A–F) contained 9.3–18.4% drug. The EE ranged from 46.48% to 92.01% (w/w) indicating that the drug loss to the aqueous phase was affected by the wall emulsion composition (or the nature of the WM) and the drug:wax compatibility, but not by the solubility of the drug in the external phases. Apparently, low drug yields of 48.35% and 46.48% were observed in preliminary studies when fatty acids such as PAc and SAc were used in formulations E and F, respectively. The inadequate dispersion of the drug in the organic phase as a consequence of the poor wetting properties of the WM resulting in the drug not getting properly encapsulated within the particles. Inefficient encapsulation of drug makes it possible for the drug crystals to remain on the outer surface of the particles. During the washing stage of the encapsulation process the drug crystals on the outside appeared to have been removed, resulting in low drug yield. Conversely, formulation D prepared by incorporation of HC, a hydrophobic emulsifier, in the organic phase had the highest EE value (92.01%) amongst all the formulations tested. HC effected encapsulation because it might increase the hydrophobicity of the wall matrix and served as a good wetting agent for the drug allowing it to be finely dispersed and enveloped in the oil phase, prior to encapsulation. Similar findings have been reported by Lewis et al. (1998) on the effect of GMS as a hydrophobic emulsifier on core retention of sustained-release diclofenac sodium wax microspheres.

The $t_{50\%}$ values of AP release from the SLS formulations (550 μm particles made of BW and WM at 1:1 ratio and a 20% theoretical loading) in S.G.F. (pH 1.2) and S.I.F. (pH 7.4) are shown in Table 1. Evidently, AP release into S.I.F. was faster than that into S.G.F. and, thus, reflected differences in extent to which the drug dissolved in the two fluids (El-Gibaly, 1998). Except for HC formulation, a maximum drug release of 43–61.3% was observed for all the formulations in S.G.F. after 4 h (figure not shown). Fastest release ($t_{50\%}$ values < 0.25 h (Table 1)) was obtained from SLS prepared with Ct.A (formulation A), PAc (formulation E) and SAc (formulation F), when compared to all of tested formulations, indicating inefficient coating of the drug and the formation of a heavily structured and macroporous matrix (SEM micrographs not shown). On the other hand, more than 85% of AP was rapidly released from these formulations within 12 h in S.I.F. and complete release occurred in about 24 h. The modifiers, which have hydroxyl or hydroxy acid groups, make the matrix more susceptible to hydration in the dissolution medium and thus provide a hydrophilic pathway for water molecules to access the drug and increase the rate of dissolution (Adeyeye and Price, 1994; Vilivalam and Adeyeye, 1994).

Although HC has excellent ability to form SLS, its formulation (code D) had very slow dissolution profiles, with no greater than 3% of the drug released after 12 h in any of the dissolution media (Table 1). In this study, HC inhibited the drug release because the emulsifier improved the efficiency of the emulsification process and finely dispersed the drug in the organic phase, causing the drug to embed deeply within the waxy matrix and thus impeding its release. In addition, the effect of HC on drug release could be attributed to its influence on the diffusion of release medium through the matrix. Such an influence could be explained in light of an overall increase in the hydrophobic nature of the modified wall matrix and thus decreased, with increase in HC content, the rate at which aqueous solutions diffused through this matrix. In a similar fashion, lipospheres containing various hydrophobic emulsifiers (such as

glyceryl esters of fatty acids (GMS, GMS dilaurate and glyceryl tristearate) and hexadecanol) retarded the rate of dissolution fluid into the matrix, consequently retarding drug release (Giannola et al., 1993; Giannola and De Caro, 1997; Lewis et al., 1998). In contrast, the use of St.A (formulation B) and Cs.A (formulation C) SLS resulted in intermediate drug release with $t_{50\%}$ values of 1.04 and 0.385 h and 12.5 and 2.5 h, in S.I.F. and S.G.F., respectively (Table 1) and a cumulative amount released of about 80% after 12 h in S.I.F. It is noteworthy that the significantly different dissolution profiles can thus be attributed to the physical and chemical properties of the respective WM which influence the gastroresistant characteristics of the material used for the drug entrapment. Therefore, BW/St.A blend was chosen for evaluation of the influence of other variables such as BW/WM ratios and HC concentration on SLS properties (Table 1). Generally, the burst release of AP from SLS prepared at various BW/St.A ratios was in the range of 42.13–51.26% in pH 7.4 buffer solution (figure not shown), which is a result of drug positioning near the outer surface or on the surface of a loose weak matrix structure of the SLS (Demirel et al., 2001). It was also observed that increasing the St.A content from 33.33% to 50% (formulations H and B, BW/WM ratios of 2:1 and 1:1, respectively) significantly ($P < 0.05$) decreased the drug release, but did not affect ($P > 0.05$) the size distribution ($d_g = 646$–$759\,\mu m$, $\sigma_g = 1.4$) and core retention of AP (EE values ≈ 67%, w/w) of the formed particles. Thus, the use of 1:1 and 2:1 BW/St.A ratios resulted in dissolution profiles with $t_{50\%}$ values of 1.04 and 12.5 h and <0.25 and 1.01 h in S.I.F. and S.G.F., respectively and about 80% drug release after 12 h in S.I.F. (Table 1). This result is an indication that the addition of St.A into wax solution greatly improved the properties of the resultant SLS, suggesting the preeminence of the influence of St.A as a wax matrix modifier over its hydrophilicity factor. These findings are not in agreement with those of Adeyeye and Price (1994) and Giannola and De Caro (1997) who reported that rapid drug (such as phenytoin and diclofenac sodium) release from St.A wax microspheres would not be unexpected due to the hydrophilicity and leaching characteristics. The discrepancy in results was due to the nature of the drug used and the encapsulation process.

To modify the properties of BW/St.A formulations to be suitable for sustained-release oral use, it was necessary to incorporate HC, as a WM and hydrophobic emulsifier at different concentrations (2.5, 5 and 10, expressed as % (w/w) of the wax mass) in the waxy matrix (Table 1). In general, spherical and free-flowing microparticles (in the d_g size range of 442–576 μm with σ_g of 1.3–1.41) were formed as a product of the selected WM combinations (codes I–K). The 550 μm particles had higher EE values of about 81% than those prepared without HC (EE value ≈ 67%). As expected, results revealed a marked reduction in the burst release and the rate of drug release from AP formulations with increased amounts of HC. When the HC concentration changed from 5% to 10% (formulations J and K), the $t_{50\%}$ values of desired SLS formulations more than doubled from 2.34 h in formulation J to 5.89 h in formulation K (Table 1) and drug release was incomplete after 24 h for all formulations. This is an evidence that some of the drug was still trapped within the matrix and the release was therefore expected to be regulated by selecting an appropriate mixture of two or more materials.

In order to gain further insight into the effects of HC (factor X_1) and other important process variables (such as PF-68 concentration (factor X_2) and drug loading (factor X_3)) on the properties of SLS formulations, a 2^3 factorial design model was used to optimize the formulations. Formulation K prepared with 10% HC concentration can offer a starting basis for this purpose since it represented a more meaningful AP content in the formulation and yet demonstrated the lowest release rates (Table 1). The model was preferentially applied to the evaluation of the SLS properties as follows.

3.1.1. Drug EE of the SLS formulations

Drug loadings ranging from 16.16% to 28.2% were achieved and were affected by initial drug content and wall emulsion composition (Table 2). In general, for a given wall system, core content was proportionally related to size of SLS. The actual drug contents for the different SLS sizes (120, 200, 550 and 655 μm) of formulation L2 were 11.77 ± 0.2051, 15.56 ± 0.4131, 17.26 ± 0.7780 and 19.48 ± 0.1981% (w/w). The specific surface areas calculated from these diameters were 5×10^{-2}, 3×10^{-2}, 1.09×10^{-2} and $0.916 \times 10^{-2}\,cm^2/cm^3$, respectively. Larger particles have a smaller total surface area per unit mass and, consequently, a lower amount of AP diffuses in the external aqueous phase during the preparation process and washing stages. As a result, the content of the drug

in larger particles is higher. In all cases, high EE values ranging from 80.8% to 92.67% of the theoretical formulation amount were accomplished. The EE increased with increasing theoretical drug loading because of the poor water solubility of the drug, and therefore, on a relative scale, a smaller loss of drug with increasing theoretical loading (Bodmeier et al., 1992). However, the limited drug loss could be mainly attributed to its extraction from peripheral parts of SLS particles during the early stages of the microencapsulation process, prior to congealing and, to a lesser extent, to losses during the washing stages. Although incorporation of HC efficiently retained the AP in the SLS formulations as mentioned before (Table 1), core retention was not affected by the proportion of HC included in the core-in-wall suspension (Table 2). It was possible that as all of the EE values of AP in SLS formulations prepared by using 2^3 factorial design were higher than 80%, the effect of HC content on drug loading was not so critical, as observed previously in preliminary studies. Formally, according to the ANOVA test (data not shown), PF-68 and initial drug load in their high levels enhanced the EE of SLS significantly ($P < 0.05$, F ratios = 10.14 and 12.29, respectively). However, the PF-68 concentration cannot be taken into account by itself since the negative interaction between the PF-68 concentration and the presence of HC at high level (X_1X_2) was observed with statistically significant differences ($P < 0.05$, F ratio = 22.08).

3.1.2. Particle size measurements

If a drug substance has a low aqueous solubility, decreasing the particle size leads to an improvement in bioavailability (Sjöström et al., 1993). Therefore, a target size (<200 μm) that is within acceptable size limits for coarse suspensions (Lewis et al., 1998) was set because the microparticles were also to be subsequently used to formulate a sustained-release suspension.

Sieve analysis of SLS formulations L1–L8 showed that most of the isolated SLS were in the particle size range of 120–655 μm, and 12.36–22.33% of the particles (depending on processing factors) were of the size fraction 550 μm, whereas 5.98–37.25% of the SLS particles had a mean size of less than 200 μm (Table 2).

Table 2 demonstrates that the d_g of the resulting eight formulations was mostly in the range of 324–589 μm and the corresponding σ_g values ranged from 1.26 to 1.5. Obviously, increasing the concentration of PF-68 (factor X_2) during the preparation enhanced ($P < 0.05$) the production yield of finer particles of less than 200 μm and decreased the d_g significantly ($P < 0.01$, F ratio = 53), whereas none of the other factors substantially affected the size distribution significantly (e.g. formulations L1, L2 and L7, L8). In particular, the use of 0.75 g of PF-68 in formulation L3 resulted in a decrease in d_g from 550 μm (formulation L1) to 324 μm and formation of 37.25% of SLS of desired size fraction (<200 μm). This result was entirely relevant to the higher dispersion property of the higher emulsifier concentration in formulation L3, resulting in decreasing the interfacial tension and formation of smaller particles.

For a given wall system composition, the influence of varying the emulsifier concentration (factor X_2) on the particle size distribution of the formed particles was less evident with the higher HC level (factor X_1) and/or drug load (factor X_3) (e.g. formulations L4, L7 and L8). These results indicate that the interactions X_1X_2 and X_2X_3 ($P < 0.05$, F ratios = 11.4 and 28.06, respectively) should be considered jointly in the d_g values which increased by around 28% when high drug load or HC content was used (Table 2). This could be attributed to the influence of HC (as a hydrophobic material) and drug amounts on the emulsion viscosity. At given constant dispersion conditions, prior to emulsification, higher viscosity (observed) contributed to formation of larger droplets of the core-in-wall suspension (Lee and Rosenberg, 2001).

The stirring rate at elevated temperature prior to cooling also had great effect on the particle size distribution. Although, the optimum stirring speed to obtain reproducible particle size (d_g = 324 μm) and yields was 400 rpm, SLS batches with a d_g of 260 μm and higher percentage (>65%) of smaller particles of desired size fraction (<200 μm) were observed when the stirring rate was increased to 600 rpm.

3.1.3. In vitro dissolution characteristics

The results of a 12 h in vitro release study from formulations L1–L8 (550 μm-particles), conducted in pH 1.2 and pH 7.4 buffer solutions at $37 \pm 0.5\,^{\circ}C$ are depicted in Table 2 and Fig. 1. Generally, the initial burst release of AP from the SLS formulations (codes L1–L8) prepared at various HC concentrations is shown in Fig. 1, in the range of 12.29 ± 1.82–26.45 ± 0.643% in pH 7.4

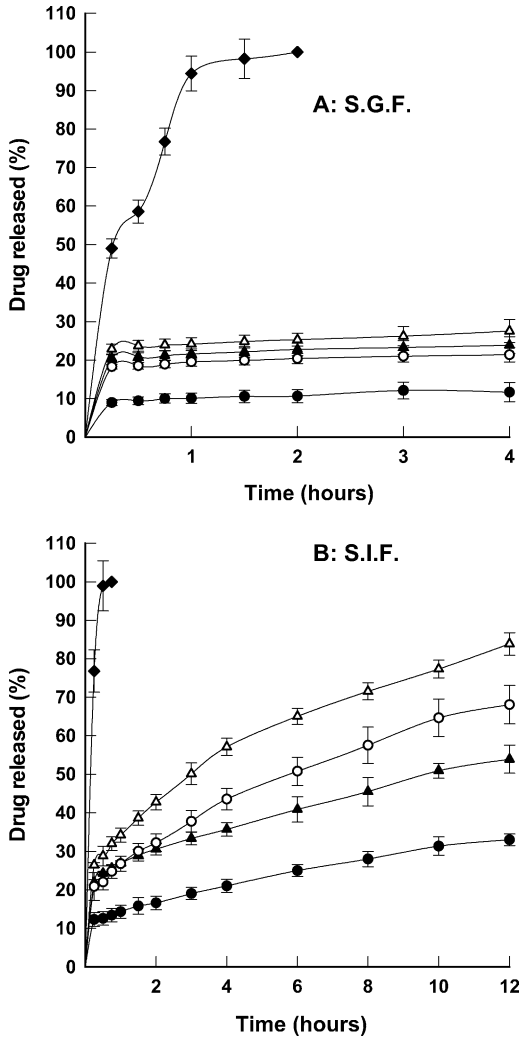

Fig. 1. Effect of HC concentration on drug release from SLS formulations (mean size: 550 μm) in (A) S.G.F. (pH 1.2) and (B) S.I.F. (pH 7.4). Drug:wax ratio (1:4): (◯) 10% (w/w) HC (LI); (●) 15% (w/w) HC (L2). Drug:wax ratio (1:2.29): (△) 10% (w/w) HC (L5); (▲) 15% (w/w) HC (L6); (♦) free drug. SLS formulations were prepared using 0.25% (w/v) PF-68 in the external aqueous phase.

buffer solution, which depended on the formulation. Obviously, the burst effect of these formulations (codes L1–L8) was lower than that of SLS formulations (codes A–H (Table 1), burst release value = 42.13 ± 2.31–51.26 ± 3.22%) prepared without HC, which is a result of efficient coating of the drug and the hydrophobicity of the matrix. A maximum drug release of only 33.01–83.84% during the 12 h release period was recorded for the SLS formulations. Except for L5 formulation, none of the formulations released their entire drug content over the 24 h release period. There was a tendency for the amount of AP released in S.I.F. to be higher than that in the S.G.F. at a given interval, regardless of processing parameters.

The effect of HC concentration (factor X_1) on the release rate of AP-loaded SLS (prepared with different drug:wax ratios) in S.G.F. and S.I.F. is also given in Fig. 1. An initial faster release was found for pure AP when compared to SLS formulations. Clearly, an increase in HC concentration from 10% to 15% (w/w) in the wax blend retarded the burst release of AP by about 48% and caused a great decrease in the cumulative % released, indicating the formation of a compact matrix structure. Using formulations prepared with a 1:4 (drug:wax) ratio, about 68.07% of the drug was released with 10% HC (code L1), while 33.01% was released with 15% HC (code L2) at the end of 12 h in S.I.F. A similar relationship was observed for the 1:2.29 drug:wax ratio formulations (codes L5 and L6). However, the rapid drug release from these formulations was ascribed to their higher drug content (Table 2 and Fig. 1). The data obtained from the study of the polynomial equation of variables ($Y_1(t_{50\%}) = 11.143 + 5.7214X_1 + 0.06963X_2 - 3.493X_3 - 0.8404X_1X_2 - 2.473X_1X_3 + 1.0654X_2X_3 + 0.6939X_1X_2X_3$) and from the statistical analysis show that factor X_1 significantly enhanced the $t_{50\%}$ values ($P < 0.01$, S.I.F.) (Tables 2 and 3). The $t_{50\%}$ for formulation L1 was 5.89 h, whereas the value for formulation L2 was 25.36 h indicating that increasing concentrations of HC resulted in an increase in resistance to diffusion of aqueous solutions through the wall matrix and increased $t_{50\%}$ values.

The variations in the release properties of the microparticles can be verified by the SEM micrographs shown in Fig. 2. SEM micrographs revealed that SLS formulations prepared with 5% HC were less spherical and had a rough and undulating surface (Fig. 2A). Numerous drug crystals and bundles of AP needles could be seen on the outer surface (before dissolution) or impregnated in a loose and porous weak matrix structure, resulting in a rapid release rate of drug. Generally, the SLS formulated with higher concentrations of HC appeared more spherical and the surface roughness was reduced (Fig. 2B–D). The surface of SLS prepared using 10% HC (formulation L1) had some of the drug

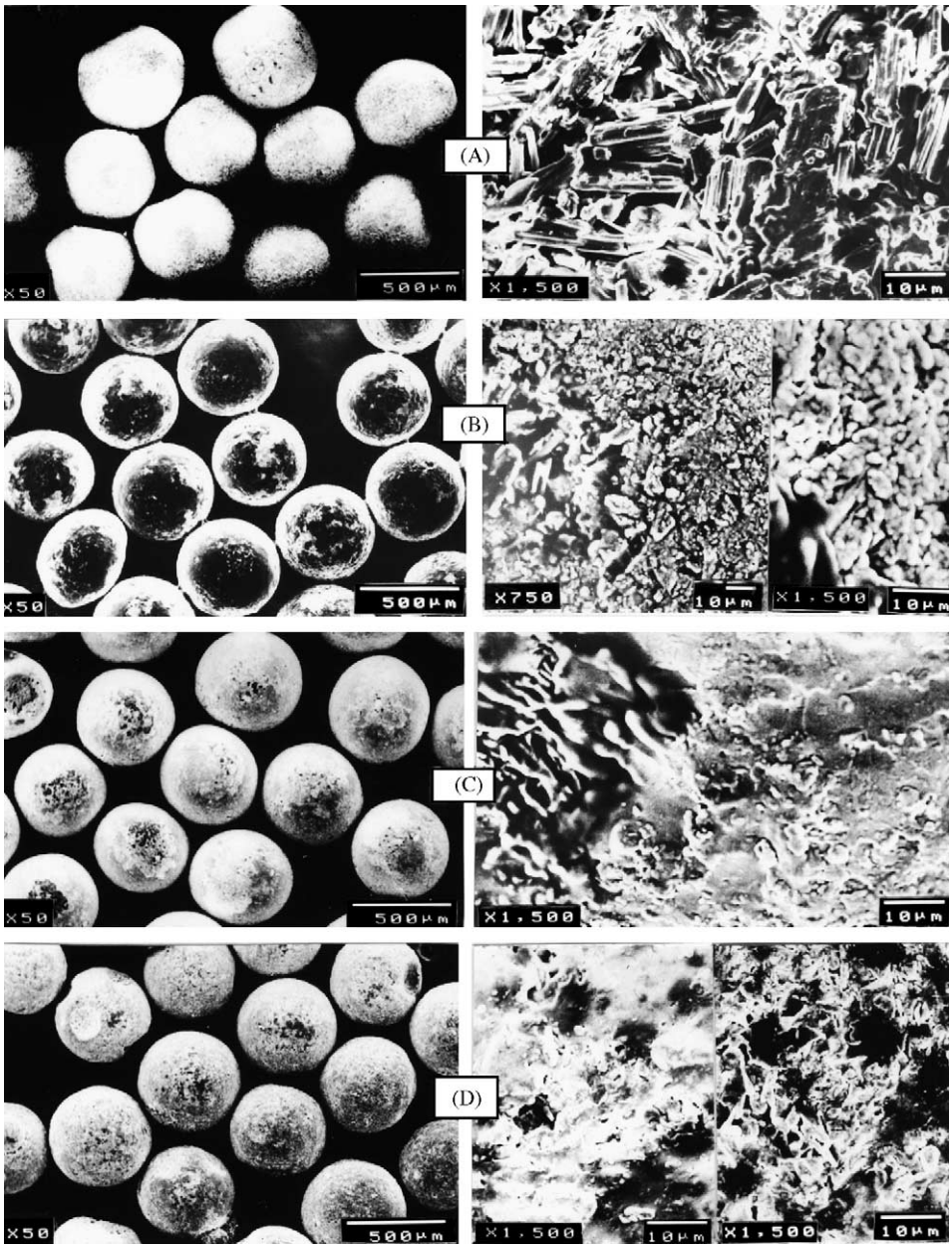

Fig. 2. SEM micrographs showing outer topography of AP-loaded SLS formulations (mean size: 550 μm) prepared with varying HC concentrations. (A–C) Pre-dissolution SEM micrographs of SLS prepared using 5%, 10% and 15% (w/w) HC, respectively; (D) post-dissolution (S.I.F., pH 7.4 for 24 h) SEM micrographs of SLS prepared using 15% (w/w) HC. SLS formulations were prepared using 0.25% (w/v) PF-68 and drug:wax ratio of 1:4; SEM micrographs A, B and (C and D) refer to formulations L, L1 and L2, respectively.

crystals, whilst those made with 15% HC (formulation L2) were less porous and appeared to maintain their shape without any drug crystals on their surfaces. These findings imply that core crystals were physically entrapped and embedded throughout a hydrophobic compact matrix structure of formulation L2 (Fig. 2C), resulting in hindrance of passive diffusion from the wall matrix and a slower core release. This accounted for the lower burst release (12.29 ± 1.82%) of AP from formulation L2 (Fig. 1). Therefore, the HC concentration must be carefully controlled in the preparation of HC microparticles using this method. A comparison of the microphotographs of the SLS morphology prior to and after a 24 h dissolution in S.I.F. revealed a porous matrix in which a multitude of voids and consequent increase in channels to the SLS surface, from which core has been released, were evident (Fig. 2C and D).

A change in theoretical drug loading from 20% (drug:wax ratio 1:4) to 30.39% (drug:wax ratio 1:2.29) caused a significant change in the release profiles of SLS formulations in S.I.F. ($P < 0.01$) (Tables 2 and 3 and Fig. 1). As anticipated, the $t_{50\%}$ values for 20% (formulation L2) and 30.39% (formulation L6) drug loadings at 15% HC concentration were 25.36 and 9.91 h, while the percentages released after 12 h were 33.01% and 53.91%, respectively (Fig. 1). The faster dissolution with increased drug load can be explained by drug crystals formation outside the SLS (micrographs not shown) and presumable increase in channels and number of pores (produced by the drug release) within the wax matrix. An effect that caused a decrease in diffusion pathlength through the released part region and enhanced drug release (Akiyama et al., 1993; Adeyeye and Price, 1994; Lewis et al., 1998). There was less difference in the dissolution profiles of formulations L1 and L5 (drug:wax ratios: 1:4 and 1:2.29, $t_{50\%}$ values = 5.89 and 3.12 h, respectively) made with 10% HC (Table 2 and Fig. 1). Opposite results were previously reported (Kim and Lee, 1992; Varshosaz and Keihanfar, 2001; El-Gibaly et al., 2003). They observed that the greater the content of microparticles, the slower was the rate of release of drug from these microparticles. The possible statistically significant ($P < 0.05$) negative interaction to take into account is the HC concentration together with the drug load in their higher levels (X_1X_3) in the formulation, which produced a decrease of approximately 61% in the $t_{50\%}$ values compared to the individual factor (X_1) (Tables 2 and 3).

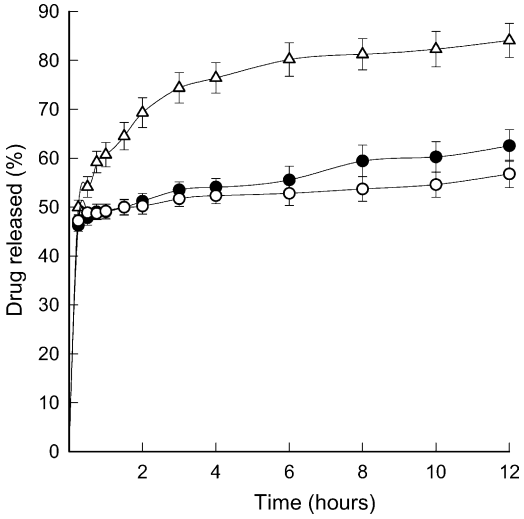

Fig. 3. In vitro release of AP from 120 μm particles (SLS formulation code L2, 11.77 ± 0.2051% (w/w) drug content) intended for use in a suspension formulation. Dissolution media: (◯) distilled water; (●) S.G.F. (pH 1.2); (△) S.I.F. (pH 7.4). See Table 2 for code L2 key.

While a slower drug release was noted with higher PF-68 concentration (formulations L7 and L8; a 1:2.29 drug:wax ratio), the ANOVA test generally revealed no significant effect for PF-68 amount as an individual factor (X_2) on drug release (Tables 2 and 3). This factor was only important to decrease the particle size at the preparation step (Table 2). On the other hand, the dissolution profiles of, for example, SLS formulation (code L2 (mean size: 120 μm); 11.77 ± 0.2051% (w/w) drug content) revealed that the cumulative amount of AP released in the different media (water, S.G.F. and S.I.F.) after 12 h were 56.78%, 62.55% and 84.08%, respectively and drug release was not completed (61–87%) after 24 h (Fig. 3), indicating that they are suitable for formulations into a sustained-release suspension. Additionally, all the formulations with HC conformed the USP23 requirements for sustained-release and enteric preparations (not greater than 30% drug release in pH 1.2 by 1.5 h, 35–60% by 4 h and 55–80% by 8 h) where 11.68–27.54% of their payload released in pH 1.2 within 4 h.

It is known that the pattern of release of active agent from the microparticles depends on the particle size of the core material (Genc et al., 1998). Therefore, SLS with a particle size range of 120–655 μm (specific surface area = 5×10^{-2}–0.916×10^{-2} cm^2/cm^3)

were utilized. The influence of the fraction size of SLS on burst release of AP from formulation L2 and the drug release rate was positive (Fig. 5). The burst release was in the range of 9.12 ± 1.33–$16.81 \pm 2.26\%$, which depended on SLS size. The $t_{50\%}$ values for the different sizes (120, 200, 302.5, 427.5, and 550 μm) were 0.25 ± 1.65, 4.12 ± 0.985, 8.52 ± 1.823, 14.38 ± 0.6571 and 25.36 ± 3.461 h, respectively. Higher burst release and release rate constants from smaller SLS were thus expected due to larger surface area and the decreased diffusion path length and wall thickness of SLS (Adeyeye and Price, 1994; Genc et al., 1998).

3.1.4. Evaluation of release kinetics

To understand the physical state of the drug inside the SLS (which has an influence on release kinetics), X-ray analyses were performed on intact drug, drug-free SLS and the representative SLS formulation (code L2) with $17.26 \pm 1.393\%$ (w/w) drug load (Fig. 4a–c). The X-ray diffractogram of drug-loaded SLS showed the presence of crystalline peaks of AP and the data of wax (Fig. 4c), indicating that the drug retains its crystalline state within the SLS and is present as a dispersion within the wax matrix.

Drug release kinetics from the SLS formulations L1–L8 were examined at pH 7.4 (S.I.F.). The release curves for AP showed a non-linear drug dissolution pattern (Figs. 1 and 3) and the drug release from the SLS was affected by the drug content and the SLS size, in addition to the structure of the matrix (Table 2 and Figs. 1–3 and 5). The analysis of the release mechanism by means of the zero-order equation (fraction released versus time) did not show either linear plots or good correlation coefficients in most of the cases (Figs. 1B and 3).

Because of the insolubility of the matrix, drug dissolution from the microparticulate system resulted in particles of the same spherical shape to spheres from which the drug had been leached and the appearance of the SLS did not change except for the holes derived from the released drug (Fig. 2C and D). The paddle speed (50 and 75 rpm) in the dissolution test did not affect the overall release rate, indicating that boundary layer effects and external diffusion were negligible. Moreover, the particle size did not change before and after complete exhaustion of the drug, suggesting that the reaction at the internal surface was not the rate-limiting step. This limiting step predominates when the drug release from a spherical particle containing a drug whose reaction site becomes recessed as the reaction proceeds, while the exact initial geometric shape is maintained at all times and the dimensions decrease in proportion to one another (Akiyama et al., 1993). All these results indicate that the release process may be an internal diffusion-limited one. Therefore, the results obtained from dissolution tests were applied to different diffusional controlled release kinetics namely, Ritger–Peppas model, Jander's equation and Baker–Lonsdale model (Baker and Lonsdale, 1974) (Table 4 and Fig. 5).

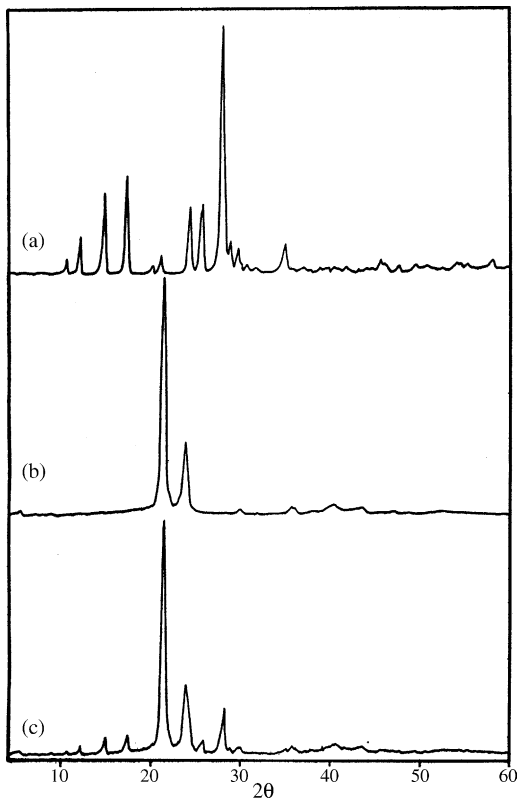

Fig. 4. X-ray diffractometric analysis of (a) crystalline AP; (b) blank SLS formulation (code L2, 0% (w/w) drug content); (c) AP-loaded SLS formulation (code L2; $17.26 \pm 1.393\%$ (w/w) drug content). See Table 2 for code L2 key.

Using the Ritger–Peppas model, a plot of the logarithm of the fractional solute release versus the logarithm of time (between 0.5 and 12 h) yielded a straight line with a determination coefficient (r^2) of

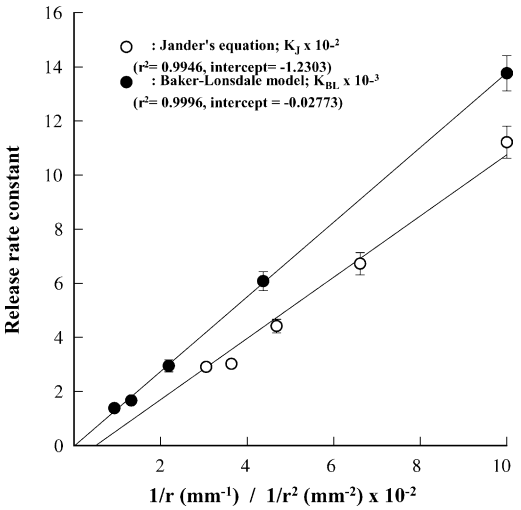

Fig. 5. Relationship between drug dissolution rate constant in S.I.F. (pH 7.4) and reciprocal of the mean radius, 1/*r* (Jander's equation) or reciprocal of the square of the mean radius; $1/r^2$ (Baker–Lonsdale model) of AP-loaded SLS formulation (code L2). See Table 2 for code L2 key.

0.9546–0.9876 for the eight formulations (Table 4). The slope of this line determines the diffusional exponent (*n*) value, which is characteristic of the transport mechanism of diffusional release. This equation may be used only for a granular inert matrix system which maintains a constant planar surface area, where the drug diffusion coefficient is clearly concentration-independent and the effect of solubility is implicit. Ritger and Peppas (1987) have shown also that two competing release mechanisms, a Fickian (non-steady state) diffusional release at $n \leq 0.43$ and a case-II transport at $n \geq 0.85$ (where the drug release rate is independent of time, i.e., zero-order release kinetics) are the limits of this phenomenon. Fickian diffusional release occurs by the usual molecular diffusion of the drug due to a chemical potential gradient. The lower values of *n* (0.2520–0.4326) computed from the Ritger–Peppas relation (Table 4) supported the expected diffusional release kinetics and also negate the erosion or solubilization of the wall matrix-driven phenomena (Ritger and Peppas, 1987; Lee and Rosenberg, 2001; Soppimath et al., 2001). In the case of polydisperse spherical systems, the values of *n* will be lower than expected and Ritger and Peppas (1987) computed values of *n* as low as 0.3 and 0.45 for Fickian and case-II transport, respectively. Further reduction of *n* values has also been reported by Soppimath et al. (2001) for nifedipine-loaded cellulose-based matrix microspheres.

In fact, the dissolution process of a solid material as a heterogeneous reaction in which the apparent shape of the solid material is not changed during the process is usually expressed by Jander's equation (Jander, 1927). This equation considers the change in the interfacial area where the release of a solid material occurs and can be applied to the internal diffusion limited process of the reaction of the sphere whose dimension does not change during the reaction and release rate is not affected by the external agitation. The diffusional controlled drug release process can also be described by the Baker–Lonsdale model for a homogeneous spherical matrix, which contains the drug in excess of the solubility in the matrix (Baker and Lonsdale, 1974). This model is also valid for a heterogeneous matrix containing capillaries or cracks which mainly contribute to the diffusion of drug dissolved in the penetrating solvent within a spherical matrix having higher drug concentration compared to the drug solubility in the release medium. The resulting SLS contained large drug crystals in relation to the size of the SLS (Figs. 2 and 4), which may be considered as a granular matrix system. The maximum determination coefficient (r^2) has been considered statistical parameter to designate the function with the best fit to the data. The spherical matrix model gave consistently higher values for the r^2 of all formulations (values = 0.9847–0.9981 for time periods of 0.5–12 h) than did the other release models (Ritger–Peppas, $r^2 = 0.9546$–0.9876 and Jander's equation, $r^2 = 0.9686$–0.9923) (Table 4). This result was confirmed by the intercept value calculated for all models. It was in the range of 0.8393 ± 0.1057–1.205 ± 0.1112 for the Ritger–Peppas model and 0.0110 ± 0.00078–0.0466 ± 0.0063 for the Jander's equation, whereas it was negligible (0.00184 ± 0.0013–0.00832 ± 0.00093) for the Baker–Lonsdale model (Table 4), suggesting that a linear relationship starting with the origin was found in the early stage for each formulation (figure not shown).

The release rates from the SLS formulation (code L2) of which sizes were different also showed a good fit with these equations. The slope of this line (the release rate constant) was plotted as a function of the reciprocal of radius of particles,

$1/r$ (Jander's equation) or $1/r^2$ (Baker–Lonsdale model) (Fig. 5). Straight line starting with the origin ($r^2 = 0.9996$, intercept = 0.02773) was only obtained for the Baker–Lonsdale model. In addition, the rate of drug release was inversely related to SLS size, consistent with that predicted by the suggested kinetic model (Fig. 5). This reflected the influence of the SLS size-dependent drug content, as well as the effect of the surface area-to-volume ratio, on the diffusion-driven mass transport phenomena through the wall matrix. All these findings indicated that the spherical matrix model for a heterogeneous matrix can best be used to describe the overall release pattern of a dissolved drug from a spherical SLS, according to their physicochemical properties, by diffusion through water-filled capillaries or a series of pores rather than by diffusion through the wax matrix (Fig. 2C and D). The result obtained is supported by the literatures reported on ibuprofen and diclofenac-loaded wax microspheres (Adeyeye and Price, 1994; Lewis et al., 1998).

3.1.5. *In vivo studies*

AP was designed to be an inhibitor of xanthine oxidase, the enzyme that catalyzes the biosynthesis of uric acid. Thus, AP reduces both the serum and urinary uric acid levels by inhibiting the formation of uric acid rather than by increasing its urinary excretion (Martindale, 1999). Table 5 shows amounts of uric acid in rats blood samples following oral administration of pure AP suspension and a SLS formulation (code L2). The oral administration of SLS suspensions was more effective ($P < 0.05$, % change from AP suspension group $\approx 13\%$) than the pure AP suspension in decreasing plasma uric acid levels in treated rats. The higher uric acid levels observed with AP suspension group may be attributed to metabolic problems and renal impairment that cause uric acid to be overproduced and/or unexcreted from the kidneys (Martindale, 1999). On the other hand, uric acid levels obtained with SLS formulation did not differ significantly ($P > 0.05$) from those obtained with controls, whereas those of AP suspension were higher ($P < 0.05$) suggesting that encapsulated AP was less hazardous to kidneys than pure drug. This finding can also be corroborated by the histopathological results on rats liver, as evidenced from Fig. 6. The examination of the liver in the control group (Fig. 6A) revealed normal histological appearance. Notably, the photographs (Fig. 6B) indicate that the pathological lesions were more pronounced in the liver of pure AP-treated rats. The liver showed marked hydropic degeneration of the hepatocytes at the periphery of the hepatic lobules. Besides, the hepatocytes in the center of the hepatic lobules underwent necrobiotic changes (Fig. 6B(a)). In some cases, there were extensive hemorrhages inbetween the hepatic cells associated with necrobiosis of the hepatocytes and edema in the disse spaces (Fig. 6B(b)). These events are in accordance with the findings of Tam and Carroll (1989) who reported fatal AP hepatic toxicity in human, where autopsy revealed hepatic toxic centrilobular necrosis. However, in the liver of a SLS formulation group (Fig. 6C), the pathological lesions were markedly minimized as the hepatocytes showed only slight swelling. Otherwise, the histological structure of the liver was more or less normal (Fig. 6C). Based on what have been mentioned, this remarkable effect of a SLS formulation shown on uric acid level and liver is most likely due to the modification in the dissolution rate of AP and also control of the in vivo release by the SLS, so that prolonged absorption properties with a lower AP concentration are maintained in blood over a longer time. This may result in increasing the antigout efficiency of AP and minimizing its deteriorous effects on liver and other organs.

Table 5
Plasma uric acid levels in controls, pure AP suspension group and AP-loaded SLS[a] suspension-treated rats

Parameter	Controls	AP-treated group	SLS-treated group[a]
Mean amount in blood (mg%)	2.141 ± 0.109	$2.558 \pm 0.133^{\#}$	$2.226 \pm 0.073^{*}$
Percentage decrease in uric acid	–	–	12.98

Values are mean ± S.E. for 10 rats.
[a] Formulation L2 (mean size: 120 μm).
[*] $P > 0.05$ vs. control and $P < 0.05$ vs. AP suspension group.
[#] $P < 0.05$ vs. control.

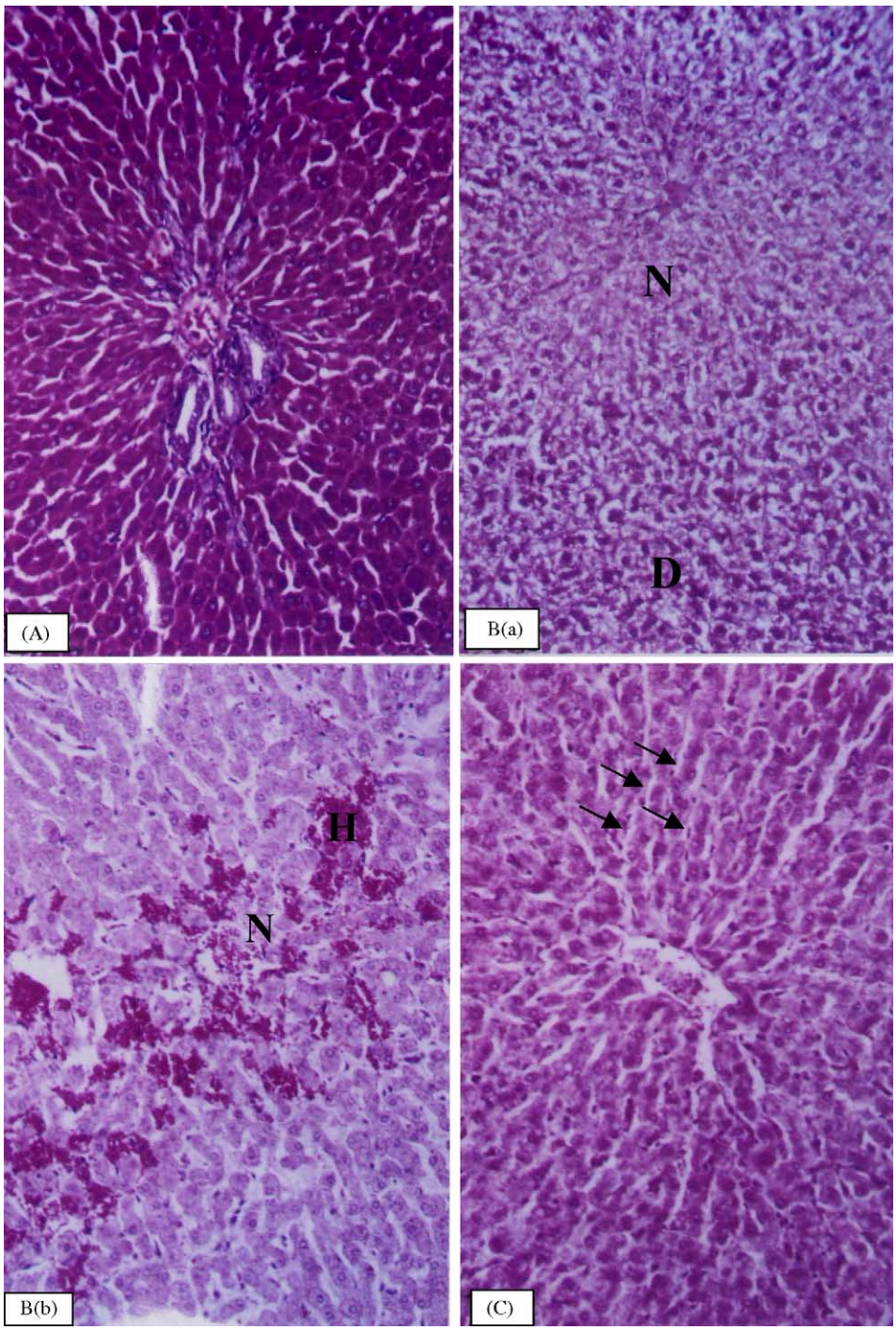

Fig. 6. Liver of (A) non-treated rats (controls), (B) pure AP-treated rats showing: (a) hydropic degeneration (D) at the periphery of hepatic lobules with necrobiosis (N) at the center of the lobules and (b) hemorrhage (H) at the hepatic parenchyma with evidences of necrobiosis (N) at the hepatocytes and (C) AP-loaded SLS-treated rats showing more or less normal hepatic structure with only minimal swelling (arrows) of the hepatocytes (formulation L2 (mean size: 120 μm); H and E stains; 10 × 10).

4. Conclusions

The modified MDP process utilized to prepare AP-loaded SLS using BW/WM blends (in a 1:1 ratio) is quite simple, rapid, inexpensive and highly reproducible. The best mixtures contained St.A and HC that are biocompatible and nontoxic materials. AP wax SLS developed by a factorial design had high EE and showed sustained-release and enteric behaviour suitable for oral use in capsules or suspensions. Increase in drug load increased the rate of drug release. The hydrophobic emulsifier (HC) played a dominant role in encapsulating the drug and enhancing the controlled release of AP from the wax matrix, whilst the hydrophilic emulsifier (PF-68), influenced the size distribution of the formed SLS. The SLS (mean size: 120 μm) obtained from optimum formulation (code L2: prepared with a 1:4 drug:wax ratio and 15% of HC), had a release profile and drug content that made them suitable for formulations into a sustained-release suspension. Overall, the slower release and enteric properties achieved may lead to prolonged activity and avoidance of hepatotoxicity, as well as, frequent administration of AP.

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