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POLYMERIC NANOPARTICLES AS POTENTIAL CARRIERS FOR TOPICAL DELIVERY OF COLCHICINE: DEVELOPMENT AND IN VITRO CHARACTERIZATION

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ABSTRACT: The aim of the present study is to develop a nanoparticle formulation as a potential carrier for topical drug delivery. Colchicine loaded Nanoparticles (Colchicine-NPs) were prepared by the double emulsion solvent evaporation method using biodegradable and non biodegradable polymers alone or as binary mixtures. Based on the evaluation of the entrapment efficiency, the particle size and the % drug released over 24 hours, a polymer mixture of poly-D,L-lactic acid (PLA), and Eudragit RL, in the weight ratio 1:1 was selected for further studies. The following formulation variables were optimized: the pH of the external aqueous phase was adjusted to a value of pH 6 in order to decrease the solubility of colchicines; the solvent composed of dichloromethane and acetone 1:1 (v/v) was selected to obtain maximum water miscibility; $CaCl_2$ was selected as a counter ion additive in the external aqueous phase; Span was used in the internal organic phase to increase entrapment efficiency and medium chain triglyceride was found to be most efficient at a concentration of 30% w/w. As a result of the optimization, the entrapment efficiency attained a maximum value of 44.6 %, the particle size was in the nanometer range and the zeta potential varied from 31.1 mV to 45 mV indicating good stability. The NPs were almost spherical with smooth morphology. The release profile of the optimized formulations was characterized by a burst release in the first 8 h followed by a sustained release up to 24 h. The cumulative percentage released after 24 h was between 15-16 %.

INTRODUCTION: Colchicine is used in the therapy of gout but it has also shown effectiveness in the treatment of skin diseases (actinic keratosis, psoriasis). Furthermore recent studies including ours [1,2] pointed out to the therapeutic effect of colchicine when patients suffering from hair loss disorder (*Alopecia areata*) were treated with topically administered colchicine film.

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Topical administration would reduce the side effects associated with systemic colchicine known to occur in majority of patients. Additionally a delivery system capable of releasing the drug over an extended period of time will achieve a prolonged therapeutic effect. Nanoparticles (NPs) as carriers for topical application present advantage allowing specific skin layers or appendages to be targeted [3] NP are taken up by cells more efficiently than micromolecules and can provide better transport and delivery of drugs. Due to their minute size and inclusion properties they can increase skin hydration and facilitate the penetration of the incorporated drug into the skin. By insuring close contact with the stratun corneum the amount of drug penetrating into the mucosa of the skin can be

increased and targeted delivery of the drug can be obtained [4]. NPs based on biodegradable and non biodegradable polymers for topical drug delivery have been investigated in recent studies [5]. But efficient incorporation of colchicine in a polymeric NP represents a challenge due to its good solubility in both aqueous and organic media.

In this study, we aim to optimize the formulation variables of colchicines-NPs in order to develop a polymer based nanocarrier which can provide a basis for topical delivery of colchicine.

MATERIALS AND METHODS:

Materials: Colchicine was purchased from El Nasr pharmaceutical chemicals company, Egypt. Polyvinyl alcohol (PVA, MW 1, 15,000) was purchased from Loba Chemie, India. Dichloromethane, Acetone, Ethyl acetate and Acetonitrile were HPLC grade and purchased from Sigma chemical company, St. Louis, USA. Eudragit RSPO and RLPO (MW150, 000), viscosity 15 mpas were gifts from Roehm Pharma Polymers, Darmstadt, Germany. Poly (D, L-lactic-co-glycolic acid) 50/50 (PLGA, MW 40,000-75,000), Inherent Viscosity 0.55-0.75 units in dl/g, Poly (DL-lactic acid) (PLA, MW 75,000-120,000), Inherent Viscosity 0.55-0.75 dl/g and poly-€-Caprolactone (PCL, Mw 14,000), Viscosity 400 - 1000 MPas were purchased from Sigma-Aldrich, USA. Span 60 and Span 85 were purchased from Fluka (Switzerland). Medium- chain triglyceride (MCT) was generously supplied by Sasol Germany GmbH, Witten, Germany. Cellulose membrane was purchased from Sigma–Aldrich, USA. Potassium dihydrogen phosphate and disodium hydrogen phosphate were of analytical grade.

Methods:

Preparation of Colchicine –NPs: Colchicine-NPs were prepared by the double emulsion solvent evaporation method [6]. 1ml of aqueous solution containing 30mg of drug was first emulsified in dichloromethane (DCM) (5ml) containing the polymers (150 mg) with an ultrasound probe for 2 min at 60 W. The resulting water-in-oil (w/o) emulsion was then poured into 20 ml of a PVA aqueous solution (1%) and emulsified by sonication for 5min at 60 W, resulting in the formation of the w/o/w emulsion. After evaporation of DCM in rota vapor for 20 min at 32º C, the NPs were isolated by centrifugation at 100,000 rpm for 30 min. The collected nanoparticles were washed two times with deionized water to remove the non-encapsulated drug and DCM trace. The supernatant was removed and NPs were resuspended in deionized water (2ml) and stored at 4^{o}C. Blank NPs were prepared in the same way.

A range of polymers was screened including biodegradable polymers, non biodegradable polymers and blends of biodegradable and non biodegradable polymers in the ratio 1/1. The formulations prepared are listed in **table 1.**

TABLE 1: SCREENING OF POLYMERS FOR COLCHICINES LOADED NANOPARTICLE

Formula	Type of polymer	Polymer Conc. (mg)
NP1	PCL	150
NP2	PLGA	150
NP3	PLA	150
NP4	PLA/PCL	150 (75:75)
NP5	RLPO	150
NP6	RSPO	150
NP7	RSPO/RLPO	150 (75:75)
NP8	RS/PCL	150 (75:75)
NP9	RL/PCL	150 (75:75)
NP10	PLGA/RS	150 (75:75)
NP11	PLGA/RL	150 (75:75)
NP12	PLA/RS	150 (75:75)
NP13	PLA/RL	150 (75:75)

PCL=poly-€-Caprolactone **PLGA=**Poly (D, L-lactide-co-glycolide); **PLA=**Poly (DL-Lactide) **RLPO=**Eudragit RLPO; **RSPO=**Eudragit RSPO

Characterization of Colchicines –NPs:

Determination of Colchicine Entrapment Efficiency: The percentage of drug incorporated in the prepared nanoparticles was calculated after separation of the nanoparticles by centrifugation. The amount of free drug in the dispersion medium was determined spectrophotometrically with UV/VIS spectrophotometer (Model 2401PC, Shimadzu, Japan). The amount of incorporated drug was determined from the difference between the initial drug content and the free drug found in the supernatant and the entrapment efficiency

(E.E.) was calculated using the following equation [7,8].

$$E.E.\ \% = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

Particle Size Analysis: Particle size analysis of colchicines-NPs was performed by laser diffraction particle size analyzer (Master Sizer X, Malvern Instruments, UK). The span values were calculated from the particle size distributions using the following equation [9]:

$$\text{Span value} = \frac{LD\ 90\% - LD\ 10\%}{LD\ 50\%}$$

Where LD 90%, LD 10% and LD 50% are characteristic diameters obtained from the distributions.

Zeta Potential (Z) **Analysis:** The (ζ) values were determined with a laser Zetameter (Zeta Sizer 2000, Malvern, UK) on 0.01 g sample placed in 50 ml double distilled water with definite electrolyte concentration at ionic strength of 2 x 10^{-2} M NaCl. The suspension was shaken for 1 hour then allowed to settle for 3 min. 10 ml of the supernatant were transferred into a standard cuvette for zeta potential measurement maintaining a constant temperature of 25°C. The zeta potential values were calculated according to Smolochowski's equation [10].

Transmission Electron Microscopy (TEM): The morphology of the polymeric nanoparticles was examined on a transmission electron microscope (Model JEM 1230, Jeol, Japan). One drop of diluted sample was deposited on the surface of a carbon coated copper grid, making sure it wetted sufficiently the surface.

***In-vitro* Release Studies:** The *in-vitro* release of colchicine from the different nanoparticles was determined in phosphate buffer pH 5.5 by the dialysis bag diffusion technique [11]. Two ml of the dispersion was placed in a cellulose acetate dialysis bag and sealed the release studies of colchicine from nanoparticles were performed at both ends. The dialysis bag was immersed in the receptor compartment containing 300 ml of the medium, which was stirred at 100 rpm and maintained at 32 ± 2°C. A 5 ml sample of the receiver medium was withdrawn at predetermined time intervals (0.5, 1, 1.5, 2, 3, 4, 5, 6, 8 and 24 h) and replaced by equivalent volume of fresh medium to maintain constant volume. The samples were analyzed for drug content spectrophotometrically at 352 nm.

The release studies were carried out in triplicates and the results were expressed as the mean values ± SD. The cumulative percent of drug released was plotted against time, the data were analyzed using linear regression equations and the order of drug release from the different formulations was determined. The release efficiency (R.E.) was calculated from the area under the release curve at time and it represents the percentage of the area of the rectangle corresponding to 100% release, for the same total time [12].

RESULTS AND DISCUSSION: A combined analysis of entrapment efficiency, particle size and drug release efficiency was used for the screening of the polymer candidates for colchicines NP. The results are shown in **Tables 2 - 4.**

Entrapment Efficiency of Colchicine-NPs: The entrapment efficiencies (E.E.) of all formulations ranged from 24.87% ± 1.09 to 30.8±0.06%.The type of polymer influenced the E.E. When biodegradable polymers were used (table 2), the E.E. ranged from 24.87% (formula NP1: PCL) to 28.67% (formula NP3: PLA). The E.E. of formulations prepared from non biodegradable polymers (table 3) was higher than that of biodegradable polymers and ranged between 28.8% (formula NP6: RSPO) and 30.8% (formula NP7: RS/RL). The E.E. of NP prepared from blends of biodegradable and non biodegradable polymers (table 4) ranged from 26.6% (formula NP8: PLGA/RL) to 28.98% (formula NP11: RS/PCL). It is evident from the results that non-biodegradable polymers ranked better than biodegradable polymers and that their addition in the polymer blends contributed to the increase in E.E. These results are consistent with those reported by Hoffart *et al*., 2002 [6] who showed that the addition of non biodegradable polymer (Eudragit RS) increased the E.E. of biodegradable polymer.

It is also apparent that in spite of the beneficial effect of non biodegradable polymers, the entrapment efficiency is still low and needs further improvement. The low E.E. can be explained by the difficulty of incorporation of the inner aqueous phase (drug) within the hydrophobic polymer matrix and reflects the tendency of the hydrophilic drug to escape to the external aqueous phase [13, 14].

Particle Size of Colchicine-NPs: The characteristic diameters (LD10%, LD50% and LD90% and the corresponding span values are shown in **tables 2 – 4**. LD 90% is used for comparison between different polymers since 90% of particles possess sizes equal or smaller than this value. It can be seen from the results that all formulations had particles sizes within the nanometer range, i.e. between 355 and 880 nm. The LD 90 % values of the NP of Eudragit polymers (480 – 570 nm, Table 3) were smaller than those of biodegradable polymers (820 – 880 nm, table 2). The LD90 % of NP prepared from polymer blends (table 4) were in between and ranged from 355nm (formula NP13: PLA/RL) to 735 ± 77.8 nm (formula NP10: PLGA/RS). The span values of all formulations were small indicating narrow size distributions and ranged from 0.7 to 1.5 (Tables 2 – 4).

TABLE 2: PARTICLE SIZE, ENTRAPMENT EFFICIENCY AND RELEASE EFFICIENCY OF COLCHICINE NANOPARTICLES FORMULATED WITH BIODEGRADABLE POLYMERS

Formula	Size (nm)					R.E. (%)
	LD90%	LD50%	LD10%	Span value	E.E. (%)	
NP1	850±14.2	500±70.7	240±0.0	1.2	24.87±1.09	8.70±0.25
NP2	880±88	520±52	290±0.1	1.1	26.63±0.15	10.50±1.11
NP3	820±82	565±63.7	325±0.1	0.9	28.67±0.06	8.90±0.60
NP4	855±7.1	440±0.0	205±0.0	1.5	27.3±0.56	6.90±0.43

TABLE 3: PARTICLE SIZE, ENTRAPMENT EFFICIENCY AND RELEASE EFFICIENCY OF COLCHICINE NANOPARTICLES FORMULATED WITH EUDRAGIT

Formula	Size (nm)					R.E. (%)
	LD90%	LD50%	LD10%	Span value	E.E. (%)	
NP5	570±28.3	310±14.2	170±0.0	1.3	29.6±0.53	12.40±0.36
NP6	480±42.4	275±21.2	160±0.0	1.2	28.8±0.06	11.90±0.40
NP7	525±35.4	290±14.2	165±0.0	1.2	30.8±0.06	13.00±0.18

TABLE 4: PARTICLE SIZE, ENTRAPMENT EFFICIENCY AND RELEASE EFFICIENCY OF COLCHICINE NANOPARTICLES FORMULATED WITH MIXTURES OF EUDRAGIT AND BIODEGRADABLE POLYMERS

Formula	Size (nm)					R.E. (%)
	LD90%	LD50%	LD10%	Span value	E.E. (%)	
NP8	730±56.6	360±14.2	185±0.0	1.5	26.6±0.12	11.20±0.22
NP9	570±14.2	305±7.1	170±0.0	1.3	27.3±0.52	10.80±0.31
NP10	735±77.8	365±35.4	175±0.0	1.5	28.9±0.58	10.90±0.43
NP11	555±21.2	265±0.1	165±0.0	1.3	28.98±0.74	10.20±0.49
NP12	560±56.6	310±14.2	170±0.0	1.3	28.4±0.44	10.25±0.53
NP13	355±0.1	300±0.0	170±0.0	0.7	27.1±0.86	11.90±0.18

It is evident that using biodegradable polymers led to nanoparticles of larger particle size compared to those using Eudragits or blends of polymers. This can be explained to be due to difference in chain lengths and in viscosities of the dispersed phase [15]. Similar findings were observed in a study by Hoffart *et al*., 2002 [6] on heparin loaded NP using biodegradable polymer (PCL and PLGA), Eudragit (RL and RS) and their blends. The authors showed that formulations prepared with Eudragit had smaller particle size compared to those of biodegradable polymer and blend of the polymers and attributed this result to the inner organic phase viscosity, the lower viscosity of the dispersed phase leading to smaller particle diameter.

***In vitro* Release Studies:** The % of released drug was measured over 24 hours in order to generate the dissolution profiles. The cumulative % released after 24h was generally low and ranged from 8.69% to 12.35% for NP from biodegradable polymers and from 14.19% to 15.97% for Eudragit based NP. The % released for NPs prepared from polymer blends was similar and ranged from 11.4% (formula NP11: PLGA/RL) to 15.27% (formula NP5: PLA/RL). Generally the release rate of NPs prepared from a blend of biodegradable and non biodegradable polymer was higher than that of biodegradable NPs and more or less equal to that of Eudragit NP. These results are opposite to those obtained by *Hoffart et al*., 2002 6 who reported that by addition of Eudragit to the biodegradable polymer, the drug release rate was decreased. They explained this to be due to the strong interaction between the polymer and the drug they used, which is difficult to disturb at the pH of the experimental conditions.

The release efficiencies, R.E. (%) were calculated from the dissolution profiles as described in experimental section 5. The R.E. at 24 h are added in tables 2 – 4 from which it can be seen that the R.E. values obtained for NP based on Eudragits **(Table 3)** ranked higher than those of NP based on polymer blends **(Table 4).** The smallest values were seen in the NP prepared from biodegradable polymers (table 2). Overall, the highest percentage of drug released (15.47%) was found in NP based on single polymer (formula NP5: RLPO), whereas the highest release efficiency (13.0%) was for NP based on mixture of Eudragits (formula NP7: RS/RL).

Release Kinetics of Colchicine- NPs: The release data was fitted to zero order, first order and Higuchi equations which are widely used in determining the release kinetics of nanoparticles. The release patterns of the drug generally followed Higuchi equation and this result is in agreement with other studies which reported that drug loaded NP provide a controlled release pattern following Higuchi's square root model [16].

Optimization of Colchicines-NPs Entrapment Efficiency: Based on the results of the screening study it appears that generally all formulations exhibited relatively low entrapment efficiency. Therefore further studies were planned to improve the entrapment of the drug. First the pH of the external aqueous phase was optimized to reduce the solubility of colchicine during the preparation of NP. Second, the effects of the following formulation-variables on the entrapment efficiency were investigated: the type of organic solvent (oil phase); the electrolyte added in the external aqueous phase; the surfactant in the organic phase and the addition of medium chain triglyceride. The polymer mixture of PLA and Eudragit RL (formula NP13) which showed good results during the screening (E.E. = 27%; R.E. = 12%; LD90% = 355nm), was selected for the optimization studies.

Preparation of Formulations for Optimization of Experimental Variables: Twelve different formulations (ONP1 – ONP12) were prepared as shown in **Table 5.**

TABLE 5: OPTIMIZATION OF FORMULATION VARIABLES

Formula	Surfactant in organic phase (% w/w)	NaCl (% w/w)	$CaCl_2$ (% w/w)	Organic phase	MCT (%) (weight, mg)
NP13	-	-	-	DCM	-
ONP1	-	-	-	50 %(v/v) DCM/Ethyl acetate	-
ONP2	-	-	-	50 % (v/v) DCM/acetonitrile	-
ONP3	-	-	-	50 % (v/v) DCM/acetone	-
ONP4	-	-	1	DCM	-
ONP5	-	-	2.5	DCM	-
ONP6	-	1	-	DCM	-
ONP7	-	2.5	-	DCM	-
ONP9	0.2 Span 60	-	-	DCM	-
ONP10	0.2 Span 85	-	-	DCM	-
ONP11*	-	-	-	DCM	15% (22.5mg)
ONP12*	-	-	-	DCM	30 % (45mg)

MCT=Medium chain triglyceride; **DCM=**Dichloromethane; *ONP 11 and ONP 12 contained 127.5 mg and 105 mg polymer respectively to compensate for the added amount of MCT

Evaluation of the Results of the Optimization Study:

Effect of pH on Saturation Solubility: In the double emulsion solvent evaporation (DES-E) technique, developed for hydrophilic compounds [17, 18], the hydrophilic compound is initially dissolved in the inner aqueous phase with subsequent polymer precipitation upon removal of the water – insoluble organic solvent. However, the method still suffers from low encapsulation efficiency of hydrophilic compounds [19] as was also evident from our screening study.

In contrast to lipophilic drug [20] encapsulation of hydrophilic agents remains a challenge because hydrophilic drugs partition rapidly into the external aqueous phase [21]. The pH adjustment of the external aqueous phase to reduce the solubility of the hydrophilic drug was utilized in order to improve the encapsulation of both large [21] and small [22] hydrophilic molecules. Cohen-Sela et al., 2008[23] studied the effect of pH on the entrapment of hydrophilic drug (alendronate) in PLGA nanoparticles prepared by double emulsion technique (w/o/w).

In the present study the effect of pH on the saturation solubility of colchicine was investigated in order to determine the optimal pH required to reduce the solubility of colchicine in the external aqueous phase. The results showed that increasing the pH value from 4 to 6 decreased the solubility of colchicine after which further increase of pH increased the solubility of the drug. For this purpose a 1% PVA solution with a pH close to 6 was used in the preparation of colchicines nanoparticles.

The decrease in solubility can be explained to be due to competitive hydration between colchicine and PVA (water soluble polymer) molecules for the aqueous phase. Therefore it is evident that PVA, while improving emulsion stability, will not increase the solubility of the drug in the external phase during formulation which is one of the reasons for the poor loading efficiency in the fabrication of nanoparticles [24]. Based on the solubility data, the entire fabrication process of colchicine –NPs was carried out using 1% PVA as stabilizer.

Effect of Organic Solvent on Entrapment Efficiency of Colchicine- Nps: The double emulsion solvent evaporation (DES-E) method has been used as a method of choice to encapsulate water-soluble drugs because the inner aqueous phase acts as a reservoir in which the drug is dissolved, whereas the oil phase serves as a dissolution barrier preventing drug leakage from the inner to the outer phase. However for a drug, such as colchicine, which is highly soluble in both aqueous and oil (organic) phase, the DES-E method leads to low encapsulation efficiency.

Therefore, alternative solvents were used to optimize the oil phase. Table 6 shows the effect of the type of organic solvent on the entrapment efficiency of colchicine NPs. It is evident from the table that the lowest E.E. (27%) was obtained with 100% DCM (formula NP13) but mixing DMC with 50 % (v/v) of ethyl acetate or acetonitrile, or acetone increased the E.E. to 31.4%, 34.6% and 44.6% respectively.

When DCM, a water-immiscible solvent, is used it takes longer for the polymer molecules to be exposed to the aqueous phase upon emulsification. The slower polymer precipitation rate provides an opening for the highly water-soluble drug to leak out of the organic phase into the aqueous phase resulting in the low % encapsulation. For this reason, varying the water-miscibility level of the solvent is thought to potentially have an impact on the encapsulation efficiency.

The polymer precipitates at a rate proportional to water miscibility level of the solvent, where the more water-miscible the solvent is the faster the polymer precipitates to form w/o/w nano-emulsion as a result of the faster exposure to the aqueous phase. The faster the polymer precipitates and encapsulates the drug in the process, less drug leaks out from the inner to the outer aqueous phase, which in theory should improve the % encapsulation.

Mixing DCM with 50% (v/v) of ethyl acetate, acetonitrile and acetone increased the water – miscibility level of these mixtures in the following order 100 % (v/v) DCM < 50% (v/v) DCM /ethyl acetate < 50 % (v/v) DCM / acetonitrile < (50 %

(v/v) DCM /acetone, which is attributed to the partial-miscibility of ethyl acetate and full- miscibility of acetone in water. The results shown in **Table 6** indicate that by increasing the water miscibility level of the oil phase the encapsulation efficiency of the drug increased from 27% to 44.6 %, which is more than 1.65 fold higher than the value, obtained using the standard DES-E method (100 % DCM). These results are in agreement with those reported by Cheow and Hadinoto, 2010 [14] who found that improvement of encapsulation efficiency of levofloxacin nanoparticles was obtained when water – miscibility level of the oil phase is increased.

TABLE 6: EFFECT OF ORGANIC SOLVENT (OIL PHASE) ON ENTRAPMENT EFFICIENCY OF COLCHICINE-NPs

Formula	Organic solvent	Entrapment efficiency (%)
NP13	100 % (V/V) DCM	27.00±0.85
ONP1	50% (V/V)DCM-Ethyl acetate	31.40±1.04
ONP2	50% (V/V)DCM-Acetonitrile	34.60±0.66
ONP3	50 % (V/V) DCM- Acetone	44.60 ±0. 67

Effect of Electrolyte on Entrapment Efficiency of Colchicine-NPs: A number of studies [25] pointed to the use of $CaCl_2$ or NaCl as counter ion in order to reduce drug solubility in water and its escape to the aqueous phase. Cohen-Sela et al., 2008[23] reported that entrapment efficiency of alendronate increased from 4.5 % to 83.5% in presence of $CaCl_2$. In the present study NaCl and $CaCl_2$ were selected as representative electrolytes to be added in the external aqueous phase and their effect on the entrapment of colchicines is shown in **Table 7.** It is evident from the table that addition of both electrolytes improved significantly the entrapment efficiency of colchicine in the NPs. The maximum effect is observed with addition of $CaCl_2$ at a concentration of 1 %. The results are in agreement with data reported by other authors. Brodin *et al*., 1978 [26] reported that a decrease in the diffusion coefficient of naltrexane hydrochloride of 73% was obtained with 9% (w/v) NaCl dissolved in the internal aqueous phase of w/o/w emulsion. Sorbitol also caused a decrease in the diffusion coefficient but its effect reached the maximum level at about 6% (w/v).These results also indicate that factors other than osmotic gradients are affecting the passage of the drug. They also suggest that NaCl competes with surfactant for water molecules at the inner w/o interface, which would result in a rigid interfacial layer which could be a more effective mechanical barrier to drug transfer. Uchida et al., 1996[25] proved that addition of electrolytes such as NaCl or $CaCl_2$ into the external aqueous phase significantly improved entrapment efficiency of brilliant blue.

TABLE7: EFFECT OF ELECTROLYTES ON ENTRAPMENT EFFICIENCY OF COLCHICINE-NPs

Formula	Electrolyte concentration (%)	Entrapment efficiency (%)
NP13	0.0	27.10±0.85
ONP4	1.0 ($CaCl_2$)	34.90±1.04
ONP5	2.50 ($CaCl_2$)	33.80±0.85
ONP6	1.0 (NaCl)	32.50±0.31
ONP7	2.50 (NaCl)	33.60±1.30

Effect of Surfactant in Organic Phase on Entrapment Efficiency of Colchicine- NPs: Generally, the characteristics of nanoparticles such as their particle size morphology, drug content and in vitro release profiles are affected by the type and concentration of surfactants in the inner and the outer water phase. The surfactants play a double role in the emulsions: as film former and barrier to the drug release at the internal interface and as a steric stabilizer at the external interface. For the optimization of this variable we employed both hydrophobic surfactants (Spans) designed to stabilize the interface of w/o internal emulsion and a hydrophilic surfactant (PVA) for the external interface of the oil globules for w/o/w emulsion. The results showed that upon addition of 0.2% of Span 60 or Span 85 in the internal organic phase the entrapment efficiency improved to about 38% compared to 27% without Spans. The results also showed that there was no significant difference however in the effect of Span 60 and Span 85 on the entrapment efficiency.

This result is consistent with the data published by Kohee and Yaghoobian, 2009 [27] who found that the encapsulation efficiency of penicillin –G increased in presence of Span in the organic phase.

Effect of Medium-Chain Triglyceride (MCT) on Entrapment Efficiency of Colchicine- NPs: MCT was used as an additive since it was shown to increase the porosity of nanoparticles rendering them susceptible to water penetration which results in swelling of the nanoparticles and increase of drug release and entrapment efficiency [28]. The effect of MCT on the entrapment efficiency of colchicines- NPs was confirmed from the results obtained in this study. It was found that adding MCT in concentrations of 15% and 30% increased the entrapment efficiency to 35.4% and 39.6 % respectively compared to 27% when MCT was not present in the formulation.

Based on the results of the optimization studies the following formulations gave the optimal entrapment efficiency: ONP3, ONP4, ONP9 and ONP12 (see table 5 for description of the formulations). The nanoparticles of the optimized formulations were characterized and their release properties were investigated.

Characterization of the Optimized Colchicine-NPs:

Particle Size Analysis of the Optimized Colchicine-NPs: The characteristic parameters LD90%, LD50%, LD10% and the span values are shown in **Table 8.** The results indicate that all the particles were in the nano size range. The LD90% ranged between 450±28.3nm and 875±7.1nm and the span values varied between 1.1 and 1.7 indicating narrow size distributions within each formulation.

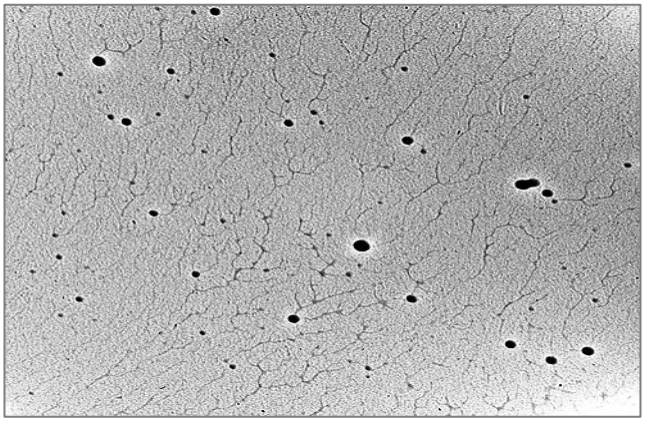
TABLE 8: PARTICLE SIZE ANALYSIS & ZETA POTENTIAL VALUES OF OPTIMIZED COLCHICINE-NPs

Formula	Size (nm)				Zeta potential (ζ)
	LD90%	LD50%	LD 10%	Span value	(mV)
NP13	555±21.2	300±0.0	170±0.0	1.3	**39.8**
ONP3	450±28.3	265±7.1	160±0.0	1.1	**45.0**
ONP4	750±14.1	380±14.1	185±7.1	1.5	**31.1**
ONP9	875±7.1	400±14.1	190±0.0	1.7	**40.9**
ONP12	670±14.1	340±14.1	175±7.1	1.5	**43.9**

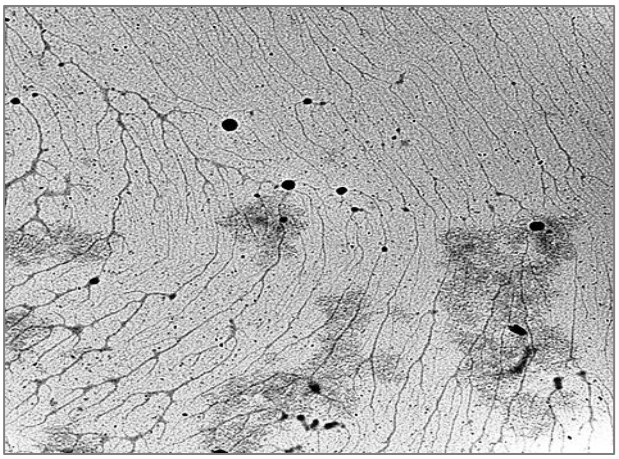
Zeta Potential Analysis (*Z*) of the Optimized Colchicine-NPs: The zeta potential (ζ) of the particles is a measure of the overall charges acquired by particles in a particular medium and is a measure of the stability of a colloidal system. The magnitude of the zeta potential has been correlated to the stability of particles and emulsion droplets. Particles will repel each other if the systems have high positive or negative value of zeta potential.

Usually, particle aggregation is less likely to occur for charged particles with high ζ (>±30 mV) due to electric repulsion [29]. The ζ values of the optimized colchicines-NPs are shown in table 8. As seen from the table, all formulations were positively charged with ζ values between 31.1 (formula ONP4) and 45.0 mV (formula ONP3) indicating good stability and dispersion quality.

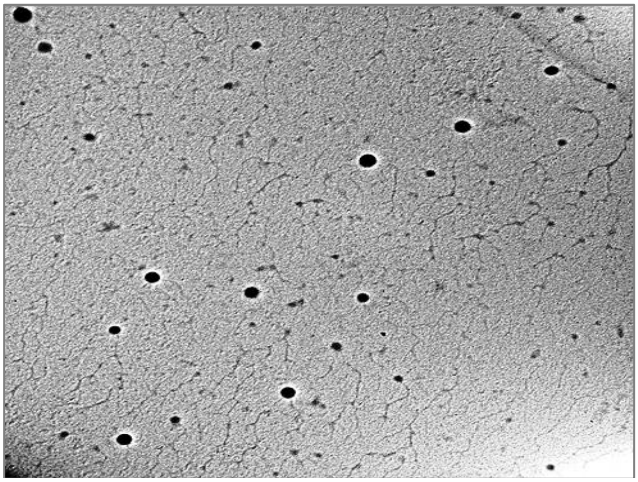
Transmission Electron Microscopy (TEM) of the Optimized Colchicine-NPs: The micrographs of the optimized colchicines-NPs are illustrated in **figure 1.** It was evident from the TEM images that the nanoparticles were almost spherical with smooth morphology and appeared as black dots, well dispersed and separated on the surface.



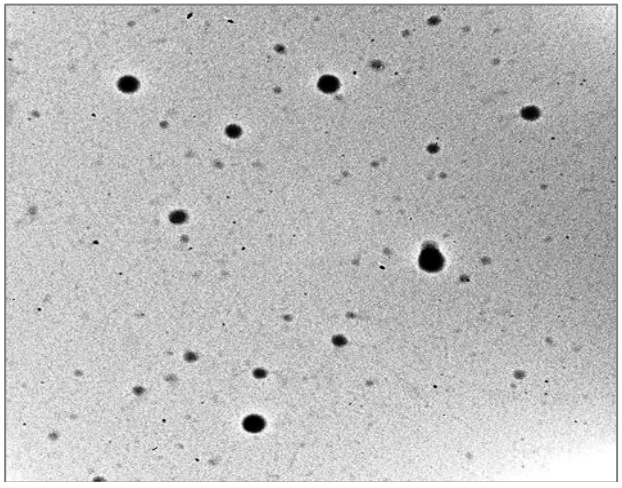
ONP4



ONP3



ONP12



ONP9

FIG. 1: TRANSMISSION ELECTRON MICROSCOPY OF THE OPTIMIZED COLCHICINE-NPs

***In vitro* Release of Optimized Colchicine-NPs Formulations:** The release profiles of the modified colchicine formulae ONP3, ONP4, ONP9 and ONP12 as well as the release profile of the original formula NP13 are shown in Figure 2. The percentage of drug released after 24 h ranged from 15 % to 16 %. It can be seen that there is no significant difference in the release rate (measured as % drug release or release efficiency) between the modified formulae.

The present results are consistent with those reported by Hao *et al*., 2002 [30] who found that the percentage of colchicine released after 24 h from niosome system was about 12 %. Also, Das *et al*., 2000 [31] found that the amount of colchicine released from PLA microspheres was about 40 % within 5 days.

It can be seen from figure 2 that irrespective of the formulation, low and biphasic release pattern was observed characterized by a burst release of colchicine during the first 8 h, followed by sustained release with constant rate up to 24 h. The initial burst release is due to the drug present on the surface or embedded in the surface layer which is released in a relatively rapid way. On the other hand, the drug incorporated into the particle core is released in a prolonged mode [32].

The release kinetics of all the optimized formulae followed the Higuchi model.

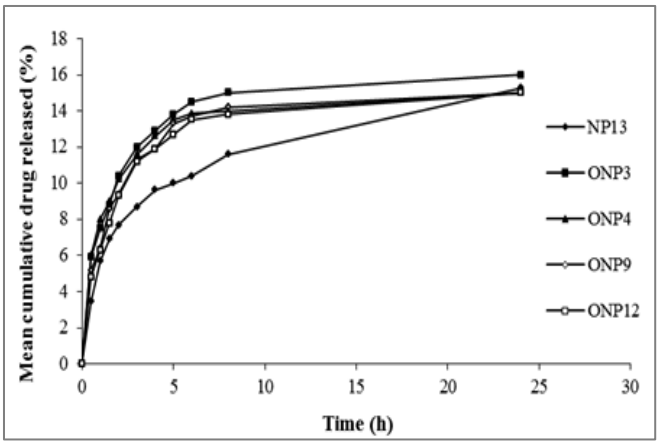


FIG. 2: *IN VITRO* RELEASE PROFILES OF OPTIMIZED COLCHICINE NANOPARTICLES FORMULATIONS

CONCLUSION: By modifying the double emulsion solvent evaporation process and the formulation variables it was possible to markedly improve the entrapment efficiency of Colchicines-NPs composed of mixture of biodegradable and non biodegradable polymers. The nanoparticles of the optimized formulations exhibited particle sizes within the nanometer range and were characterized by narrow size distribution. They appeared spherical, well dispersed and had positive charge on the surface (ζ >30 mV) indicating good stability. The maximum cumulative percentage released after 24 hours was 16%. The release profiles exhibited biphasic release pattern with fast release during the first 8 hours followed by sustained release with constant up to 24 hours. For topical release both features are of interest since burst release can improve the penetration of the drug and sustained release becomes important in supplying the skin with drug over a prolonged period of time.

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