

# Carbopol 934, 940 and Ultrez 10 as viscosity modifiers of palm olein esters based nano-scaled emulsion containing ibuprofen

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**Abstract:** Micro-emulsions and sometimes nano-emulsions are well known candidates to deliver drugs locally. However, the poor rheological properties are marginally affecting their acceptance pharmaceutically. This work aimed to modify the poor flow properties of a nano-scaled emulsion comprising palm olein esters as the oil phase and ibuprofen as the active ingredient for topical delivery. Three Carbopol<sup>®</sup> resins: 934, 940 and Ultrez 10, were utilized in various concentrations to achieve these goals. Moreover, phosphate buffer and triethanolamine solutions pH 7.4 were used as neutralizing agents to assess their effects on the gel-forming and swelling properties of Carbopol<sup>®</sup> 940. The addition of these polymers caused the produced nano-scaled emulsion to show a dramatic droplets enlargement of the dispersed globules, increased intrinsic viscosity, consistent zeta potential and transparent-to-opaque change in appearance. These changes were relatively influenced by the type and the concentration of the resin used. Carbopol<sup>®</sup> 940 and triethanolamine appeared to be superior in achieving the proposed tasks compared to other materials. The higher the pH of triethanolamine solution, the stronger the flow-modifying properties of Carbopol<sup>®</sup> 940. Transmission electron microscopy confirmed the formation of a well-arranged gel network of Carbopol<sup>®</sup> 940, which was the major cause for all realized changes. Later *in vitro* permeation studies showed a significant decrease in the drug penetration, thus further modification using 10% w/w menthol or limonene as permeation promoters was performed. This resulted in *in vitro* and *in vivo* pharmacodynamics properties that are comparably higher than the reference chosen for this study.

**Keywords:** Carbopol<sup>®</sup> resins, Ibuprofen, nano-scaled emulsion, viscosity modification

## INTRODUCTION

Recently, many researchers and investigators have reported the effectiveness of delivering various drugs through the skin via using emulsion preparations with nano-metric droplets size (usually in the range of 20-200 nm). They have proven that such dosage forms possess influential solubilization capability, thermodynamic stability, controllable droplet size, enhanced permeability, *etc* (Baroli *et al.*, 2000; Puglia *et al.*, 2006; Yilmaz *et al.*, 2006; Baboota *et al.*, 2007; Mou *et al.*, 2008).

Usually, micro-emulsions and sometimes nano-scaled emulsions show poor rheological properties with a very low viscosity making them behave as Newtonian fluids or semisolids of high fluidity. These properties render them pharmaceutically inapplicable and commercially unacceptable as topical products (Zhu *et al.*, 2009). In such circumstances, the addition of thickening agents or gel-network forming substances becomes highly recommended to overcome these minor drawbacks (Mou *et al.*, 2008).

Materials, like: Carbopols<sup>®</sup> (poly-acrylic acid polymer), gums (polysaccharides), semi-synthetic cellulosic

derivatives, *etc.*, are widely used pharmaceutically and cosmetically to achieve various purposes, such as: emulsification, stabilization and rheological control (Kim *et al.*, 2003).

Recently, Carbopol<sup>®</sup> resins have been considered extensively by many researchers and scientists, since they provide a wide range of applications as thickening agents, emulsifying agents, suspending agents and tablets compressed matrix forming agents, which is particularly used in controlling drug release (Neau *et al.*, 2000).

In general, Carbopol<sup>®</sup> polymers are polymers of acrylic acid, cross-linked with polyalkenyl ethers or divinyl glycol. They are hydrophilic, tightly coiled, mildly acidic (pka  $6.0 \pm 0.5$ ) fluffy, white and dry flocculated powders with particles having a size of about 0.2  $\mu\text{m}$  in diameter (Lubrizol Advanced Materials Inc. TDS-222, 2008). Their neutralized aqueous dispersions demonstrate a high viscosity, which makes them fairly popular for controlling the flow properties of topically applied dosage forms as they are inexpensive, transparent and harmless and easy to prepare and clean (Piau 2007).

Carbopol<sup>®</sup> polymers are commonly synthesized by cross-linking process with the specific substrate. The degree of cross-linking and the manufacturing conditions usually

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produce various grades of Carbopols<sup>®</sup>. Each grade demonstrates different criteria that fit it into a specific application (Hosmani *et al.*, 2011). Structurally, each discrete unit of this polymer appears as a network of polymer chain interrelated by cross-links. Removing these cross-linkages would render the primary particles to appear as an intertwined but not chemically bonded collection of linear polymer chains. Such linear polymers are soluble in water and polar solvents, like Carbopol<sup>®</sup> 907. On the other hand, all other types of Carbopols<sup>®</sup> are cross-linked. Because of their hydrophilicity and high water sorption ability, they can distend in water up to 1000 times their original volume and 10 times their original diameter forming a gel upon exposure to a pH environment above a range of 4-6. Once these Carbopol<sup>®</sup> molecules are dispersed in water, they start hydrating and uncoiling to a certain extent. Neutralization of the polymer carboxylate groups using an alkaline substance makes them highly ionized to form rigid gels (Lubrizol Advanced Materials Inc. TDS-222, 2008; Lu and Jun, 1998; Fresno *et al.*, 2002; Lubrizol Advanced Materials Inc. TDS-237 2009). This neutralization step is considered as the most common way to achieve the maximum thickening capability of Carbopol<sup>®</sup> polymers. It can be accomplished by adding a common base, like sodium hydroxide or organic amines, such as triethanolamine, which cause the carboxylate groups of these polymers to be converted into a salt. Upon ionization of these carboxylate groups on the polymer backbone, electrostatic repulsion amongst the negatively charged particles is observed which finally adds to the swelling and thickening capabilities of these polymers (Lubrizol Advanced Materials Inc. TDS-237 2009). In addition, several experiments have been conducted to determine the pH range that renders Carbopol<sup>®</sup> polymers to be powerful thickening and gelling agents. It was found that a pH range of 5-9 usually leads to the formation of highly viscous systems. However, pH values less than 5 and above 9 can be also used, but higher levels of Carbopols<sup>®</sup> must be employed to produce higher viscosity levels (Lubrizol Advanced Materials Inc. TDS-237 2009).

Specifically, Carbopol<sup>®</sup> 934, 940 and Ultrez 10 were reported by many to be of great applications pharmaceutically and cosmetically (Blanco-Fuente *et al.*, 1996; Lu and Jun, 1998; Fresno *et al.*, 2002; Kim *et al.*, 2003; Mou *et al.*, 2008). They demonstrate different rheological properties and viscosity values, which are mainly reflected by the polymer particle size, molecular weight between cross-links, allocation of molecular cross-links and the fraction of the overall units that arises as terminal units, i.e. free chain ends (Lubrizol Advanced Materials Inc. TDS-222, 2008). Recently, Carbopol<sup>®</sup> Ultrez 10 was introduced as a hybrid between Carbopol<sup>®</sup> 940 and 934. It shows superior dispersion properties and potentially wider range of applications. These properties make the process of producing homogenous colloidal

dispersion of Carbopol<sup>®</sup> Ultrez 10 easier, shorter and cheaper. In other words, less effort and energy are required to produce a lump-free dispersion (Welin-Berger *et al.*, 2001; Fresno *et al.*, 2002). Preliminary studies conducted by our research group showed that a nano-scaled emulsion comprising ibuprofen: phosphate buffer pH 7.4: palm olein esters: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.2 had the best physical characteristics among all other formulations. Therefore, the current work is aimed to modify the poor flow properties of this formula and assess the gel-network forming properties of three different types of Carbopol<sup>®</sup> resins. Carbopol<sup>®</sup> 934, 940 and Ultrez 10, at various concentrations were utilized to achieve the flow modification task. Furthermore, different basic substances were used to assess the swelling and gelling characteristics of the selected polymers by calculating the intrinsic viscosity of all formulae prepared. The effects of these rheology controlling ingredients on the zeta potential, appearance and droplets size were also evaluated. Finally, the modified formula was examined intensively using transmission electron microscopy and compared rheologically to the reference chosen to ensure the production of a pharmaceutically accepted nano-scaled emulsion capable of delivering ibuprofen locally.

## MATERIALS AND METHODS

### *Materials*

Ibuprofen was provided by Noveltex life-science, China. Tween 80 was purchased from Sigma-Aldrich Chemie, GmbH, Germany. Palm olein esters (POEs) oil was a gift from Universiti Putra Malaysia (co-researchers). Sodium benzoate, as preservative was purchased from Sigma-Aldrich Chemie, GmbH, Germany. Carbopol<sup>®</sup> polymers namely: Carbopol<sup>®</sup> 934 (Carb 934), Carbopol<sup>®</sup> 940 (Carb 940) and Carbopol<sup>®</sup> Ultrez 10 (Carb U10) were supplied by BF Goodrich Chemical Co. Cleveland, OH, USA. Sodium hydroxide and potassium dihydrogen phosphate were supplied by R & M Chemicals, England. Triethanolamine (TEA) was obtained from E. Merck, Darmstadt, Germany. All other reagents were of analytical grade.

### *Methods*

Initially, several experiments were conducted using various types of surfactant systems of different HLB values alongside with POEs as the oil phase so as to construct different phase-diagrams (Abdulkarim *et al.*, 2011). Thereafter, several evaluation procedures, such as measuring ibuprofen solubility in the oil-surfactant system, nano-scaled emulsion characterization and preservation studies were carried out. Subsequently, the resultant nano-scaled emulsion comprising ibuprofen: phosphate buffer pH 7.4: palm olein esters: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.2 was chosen for further investigation. The choice of this

formula was based on its tiny droplets size (20.8 nm), full transparency, high drug solubility, and optimal zeta potential (-28.16 mV) (data not shown). However, its poor rheological behavior revealed by the low intrinsic viscosity (11.47 poise) required an extensive modification to make it a pharmaceutically acceptable dosage form.

#### **Preparation of Carbopols® neutralized aqueous dispersion**

The homogenous aqueous dispersions of various types of Carbopols® (934, 940 and Ultrez 10) were prepared by adding the weighed amount of each Carbopol® gradually and slowly to the dispersion medium with continuous mixing at 400-500 rpm for 30 min. Three different concentrations of each polymer (0.50% w/w, 0.75% w/w and 1.00% w/w) were used. The dispersion medium pH was kept constant by using either phosphate buffer (PB) pH 7.4, or TEA aqueous solution pH 7.4. After the formation of a homogenous dispersion, the mixture was kept undisturbed overnight to ensure complete swelling and gel-network formation. The concentration of the selected polymer was then optimized at various pH values (6.5-8.0) by using different concentrations of TEA. This was accomplished to assess the ability of TEA to neutralize the carbonyl groups present on the selected polymer backbone, which usually can affect its gelling potency.

#### **Preparation of nano-scaled emulsions containing ibuprofen**

Preparation process was conducted by initially blending the specified ratios of the oil phase (POEs) and the emulsifier (Tween 80) in a beaker and mixing at a speed of 750 rpm using a low shear mixer equipped with three blades propeller and temperature control of  $40 \pm 1^\circ\text{C}$  for 30 min. Subsequently, 5% w/w ibuprofen was dissolved in the solution and the resultant mixture was further revolved at the same speed and temperature for another 30 min. The external phase (pH 7.4) comprising two separate proportions, a small part containing 0.20% w/w sodium benzoate as preservative and a second large one having specific amounts of Carbopol® 934, 940 and Ultrez 10, was successively added drop by drop with continuous stirring for another 90 min. Agitation using the low shear mixer at a rotation speed of 1500 rpm was then used for approximately 4-5 hr to ensure the formation of homogenous nano-scaled emulsions. The composition of the basic formula and formulae 1-9 comprising various types of Carbopols® at three different concentrations prepared using PB pH 7.4 as the external phase is shown in table 1. Table 2 illustrates the composition of formulations 10-13 which contain four different concentrations of Carb 940 with the best rheological properties prepared by using TEM pH 7.4 as the external phase.

**Table 1:** Composition of formulae prepared using different types and concentrations of Carbopol® resins neutralized by PB pH 7.4

Formulation	Carbopol® resin type	Carbopol® resin percentage w/w
Basic	None	0.00
1	934	0.50
2	934	0.75
3	934	1.00
4	940	0.50
5	940	0.75
6	940	1.00
7	U10	0.50
8	U10	0.75
9	U10	1.00

All formulations contain ibuprofen: PB pH 7.4: POEs: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.20.

**Table 2:** Composition of formulae prepared using the selected Carbopol® resins at various concentrations neutralized by TEA pH 7.4

Formulation	Carbopol® resin 940	Carbopol® resin percentage w/w
10	940	0.00
11	940	0.50
12	940	0.75
13	940	1.00

All formulations contain ibuprofen: TEA aqueous solution pH 7.4: POEs: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.20.

#### **Nano-scaled emulsions characterization**

In general, all nano-scaled emulsions prepared were evaluated in terms of zeta potential, visual appearance and physical stability, droplets size and flow properties.

#### **Zeta Potential**

Zeta potential was measured using Zeta-sizer, Nano Malvern, UK. All formulae tested were diluted with the external phase to fix the ionic strength of each formula. The samples were mixed well to get clear solution and bubbles were eliminated from the samples to prevent any change in the mobility of samples. The instrument works on the principle of Laser Doppler Electrophoresis technique to measure the electrophoretic mobility of the droplets. Zeta potential is calculated and presented automatically based on the particles mobility by the machine.

#### **Appearance and visual stability**

Visually, all formulae produced were checked for changes in appearance and initial sign of destabilization, such as phase separation.

### **Droplets size**

Droplets size measurement of the produced nano-scaled emulsions was carried out to ensure that the droplets of all formulae following the addition of various amounts and types of Carbopol<sup>®</sup> resins were still in the nano-metric range. This test was accomplished using Nanophox particle size analyzer, Sympatec GMBH, Germany and Malvern zeta sizer 1000 HAS, Malvern Works, UK. Both equipments compute and determine the globules size depending on the basic principle of photon correlation microscopy. In this technique, the Laser light beam that diffracted by the droplets is detected by an image detector which processes the data to determine and record the droplets size. The limit of detection of these equipments ranges between 1 nm and 1000 nm. The samples were diluted with the corresponding external phase to get the K count in between 50-200 as required by Malvern and Nanophox before performing the measurement. K count is a value that represents the number of particles contained in each sample. Light scattering was monitored at an angle of 90° and a temperature of 25°C.

### **Flow properties**

The rheological measurements were performed with a rheometer (Rheologica instrument AB, Sweden). A cone-and-plate geometry (plate diameter 40 mm) was used and the experiment was conducted under the following conditions: about 0.5 gm of the sample to be studied was placed on the plate and left to equilibrate at a controlled temperature (25 ± 0.1°C) for 3 min before bringing down the cone. This was done to ensure the thermal as well as the structural equilibration of all samples. Excess sample was removed using spatula and tissue papers. The shear stress was applied in an increasing manner at a rate of 5 pascal/2 sec and the shear rate measurements were recorded (Welin-Berger *et al.*, 2001). The experiment was repeated for other samples and triplicate trials were done for each of them. Rheograms were drawn by plotting shear stress on the x axis and shear rate on the y axis. Rheological parameters of the nano-scaled emulsions were calculated using the following equations (Sinko, 2006)

$$F^N = \eta^G \quad (1)$$

$$\log G = N \log F - \log \eta \quad (2)$$

Where  $G$ ,  $F$ ,  $N$  and  $\eta$  are shear rate, shear stress, exponential constant that defines the type of flow and viscosity coefficient, respectively.

### **Transmission electron microscopy (TEM)**

Selected nano-scaled emulsion comprising Carb 940 (0.50% w/w) was examined by TEM to verify or contradict the size of droplets that was measured by photon correlation microscopy and investigate the surface feature as well as the structure of the sample. TEM, Leo 912 AB Etem was used for structural and droplets size measurements. Samples were placed on a formvar carbon-coated copper grid (200-mesh/inch) and then stained with

1% phosphotungstic acid. Any excess of phosphotungstic acid on the sample was gently wiped off using filter paper and these samples were examined after drying for 30 min at room temperature.

### **STATISTICAL ANALYSIS**

The collected data was analyzed statistically using SPSS version 16. One-way analysis of variance (ANOVA) test was used to measure the significant differences between prepared formulae. Statistically, the significant difference was considered to be at ( $P < 0.001$ ).

### **RESULTS**

We have previously mentioned that the selected nano-scaled emulsion had shown poor rheological characteristics. Accordingly, in the present investigation, we successfully designed POEs-based nano-scaled emulsion containing 5% w/w ibuprofen with better flow properties compared to that of the reference selected.

#### **Zeta potential**

Zeta potential is defined as the potential difference between the shear plane (i.e. stationary layer attached to the dispersed colloidal particles) and the electro-neutral region of the solution. The importance of zeta potential comes from its close relation to the stability of colloidal dispersions. Therefore, a value of ± 25 mV can be taken as the arbitrary value that separates low-charged surfaces from highly-charged surfaces. According to Deryaguin, Landau, Verwey and Overbeek ("DLVO" theory), the zeta potential specifies the degree of repulsion between adjacent and similarly charged particles in a dispersion.

So, any change in the overall potential carried by the droplets or particles of various dispersions can be ascribed to their stability or instability (Attwood, 2004; Sinko, 2006). The addition of ionisable substances, like Carbopol<sup>®</sup> polymers can alter the total charge carried by the system. Thus, the zeta potential of every prepared formula with certain amount and type of Carbopol<sup>®</sup> resin was assessed. Carbopol-free formula (basic formula) had a zeta potential value of  $-26.72 \pm 0.11$ . The addition of different types of Carbopols<sup>®</sup> at various concentrations insignificantly changed this value.

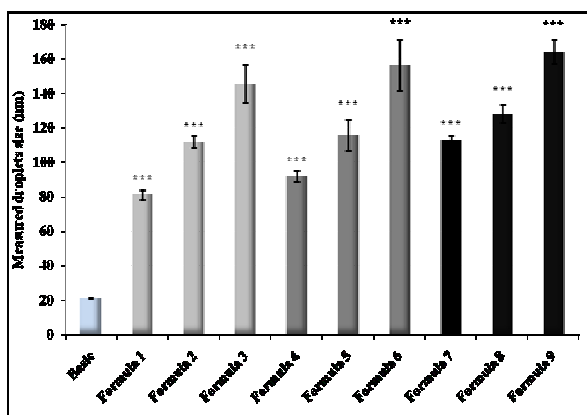
#### **Appearance and visual stability**

The addition of different Carbopols<sup>®</sup> resulted in a change in the appearance of formulae studied. A visually, clear or transparent dispersions turned to unclear or opaque ones.

#### **Droplets size and flow properties**

The collected data, presented in fig. 1, showed a significant ( $P < 0.001$ ) increase in the droplets size of all formulae prepared upon adding Carb 934, Carb 940 and Carb U10, separately at all concentrations used. It is

obvious that the highest rise in droplets size was attained by Carb U10, the universal Carbopol® polymer (Fresno et al., 2002; Lubrizol Advanced Materials Inc. TDS-222, 2008).

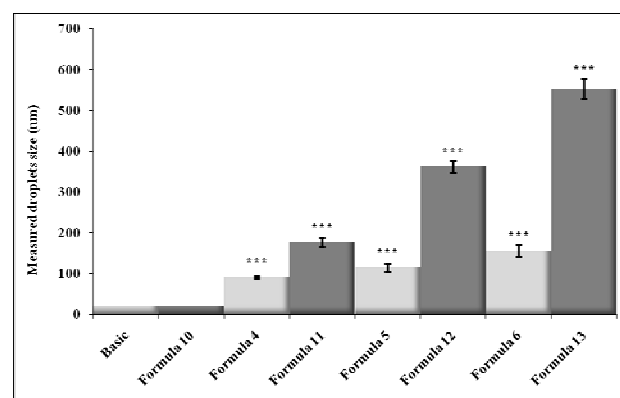


**Fig. 1:** Droplets size analysis of nano-scaled emulsions containing different types and concentrations of Carbopol® resins using PB solution pH 7.4 as the external phase. All formulations contain ibuprofen: phosphate buffer pH 7.4: POEs: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.2.

Rheologically, Carb 940 demonstrated the best rheology-modifying properties among all types used, as presented in table 3. The results revealed a significant ( $P < 0.001$ ) increase in the calculated intrinsic viscosity of formulae 2-9 compared to carbopol-free formula. The increment in the viscosity of formula 1, which contains 0.50% w/w Carb 934, was statistically insignificant from that of the basic formula. This means that the formed gel network of this polymer in the dispersion medium was insufficient to impede or increase the resistance toward the freely moving droplets. Formulations 4, 5 and 6 comprising different concentrations of Carb 940 showed also statistically ( $P < 0.001$ ) higher intrinsic viscosity than formulae 7, 8 and 9 containing Carb U10 at the same

concentration. Moreover, formulae 6 and 9 containing 1.00% w/w of Carb 940 and Carb U10 respectively showed a comparable intrinsic viscosity to that of the reference. However, the droplets size of these formulations was significantly higher than that of the basic formula, where the highest droplets size was disclosed by formula 9 as shown in fig. 1.

Data presented in fig. 2 and table 4 showed that the addition of TEA dramatically ( $P < 0.001$ ) increased both the dispersion droplets size and intrinsic viscosity. Formula 4, comprising 0.5% w/w Carb 940 and PB pH 7.4, was found to have a droplets size of about 90 nm. This size was almost doubled as revealed by formula 11 upon changing the neutralizing agent from PB pH 7.4 to TEA with the same pH value. Similarly, formulae 12 and 13 showed a three-fold increase in droplets size compared to formulations 5 and 6 as shown in fig. 2.



**Fig. 2:** Droplets size analysis of nano-scaled emulsions containing different concentrations of Carb 940 using PB and TEA solutions pH 7.4 as the external phase. All formulations contain ibuprofen: aqueous phase pH 7.4: POEs: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.2.

**Table 3:** Calculated intrinsic viscosity of nano-scaled emulsions containing different types and concentrations of Carbopol® resins using PB solution pH 7.4 as the external phase

Formulation	Type of Carbopol® resin used	Carbopol percentage w/w	$\eta_i^a$ (Poise)
Basic	No Carbopol	0.00%	11.47 ± 0.70
1	934	0.05%	18.41 ± 1.45
2	934	0.75%	24.91 ± 0.48
3	934	1.00%	39.99 ± 0.64
4	940	0.05%	31.39 ± 0.80
5	940	0.75%	56.97 ± 3.09
6	940	1.00%	161.79 ± 7.25
7	U10	0.05%	22.92 ± 0.58
8	U10	0.75%	36.51 ± 1.27
9	U10	1.00%	133.07 ± 1.88

<sup>a</sup> Calculated intrinsic viscosity. (Mean ± SD, n = 3). All formulations contain ibuprofen: phosphate buffer pH 7.4: POEs: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.20.

**Table 4:** Calculated intrinsic viscosity of nano-scaled emulsions containing different concentrations of Carb 940 using PB and TEA solutions pH 7.4 as the external phase

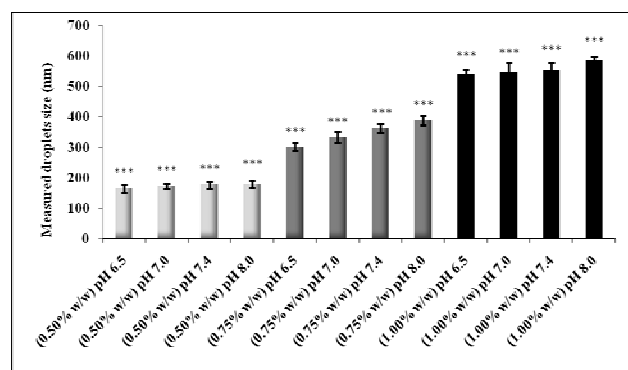
Formulation	Carb 940 percentage w/w	Type and pH of external phase	$\eta_i^a$ (Poise)
Basic	0.00%	PB pH 7.4	11.47 ± 0.70
10	0.00%	TEA pH 7.4	11.87 ± 0.72
4	0.50%	PB pH 7.4	31.39 ± 0.80
11	0.50%	TEA pH 7.4	148.00 ± 7.36
5	0.75%	PB pH 7.4	56.97 ± 3.09
12	0.75%	TEA pH 7.4	7565.41 ± 160.89
6	1.00%	PB pH 7.4	161.79 ± 7.25
13	1.00%	TEA pH 7.4	51589.81 ± 751.58

<sup>a</sup> Calculated intrinsic viscosity. (Mean ± SD, n = 3). All formulations contain ibuprofen: aqueous phase pH 7.4: POEs: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.20. Reference calculated intrinsic viscosity equals to 122.98 ± 2.44 (Poise).

Table 4 demonstrated a very notable rise in the samples calculated intrinsic viscosity. Formulae 11 and 12 in comparison with formulae 4 and 5 showed 5 times and 135 times increase in their calculated intrinsic viscosity, respectively. Likewise, formulation 13 demonstrated a remarkable rise in its viscosity of about 320 times compared to that of formula 6.

Overall, it is obvious from the data collected that the properties of Carb 940 aqueous dispersions are affected by the type of neutralizing agent used. Therefore, another study was carried out to evaluate the effect of different concentrations of TEA (various pH values) on the properties of emulsions prepared.

Several formulae with the same basic composition, previously mentioned, were prepared using an aqueous external phase with pH values of 6.5, 7.0, 7.4 and 8.0 using different concentration of TEA. The data shown in fig. 3 and table 5 demonstrate a significant ( $P < 0.001$ ) increase in both droplets size and intrinsic viscosity of all formulae as the pH increases.



**Fig. 3:** Droplets size analysis of nano-scaled emulsions containing different concentrations of Carb 940 using TEA solutions as the external phase at various pH values. All formulations contain ibuprofen: TEA solution pH 7.4: POEs: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.2.

Finally, the formula that contained the lowest concentration of Carb 940 (0.50% w/w) pH 7.4 neutralized by either PB or TEA were compared with the reference formula which contains the same concentration of ibuprofen (5% w/w).

Rheologically, this comparative study demonstrated that formula 4 failed to produce pharmaceutically acceptable properties than that of the reference, while formulation 11 succeeded. Formula 11 has significantly ( $P < 0.001$ ) higher intrinsic viscosity than that of the reference chosen for this study. Thus, it can be suggested that an o/w nano-scaled emulsion exhibiting acceptable flow properties, appearance and droplets size was successfully prepared by using an aqueous external phase pH 7.4 comprising 0.50% w/w of Carb 940, as a thickening agent, and TEA aqueous solution as a neutralizing agent.

**Transmission electron microscopy (TEM)**

Formula 11 was subsequently examined microscopically using TEM. This step was carried out to assess the structure of this formula and to confirm its droplets size previously measured using photon correlation technique.

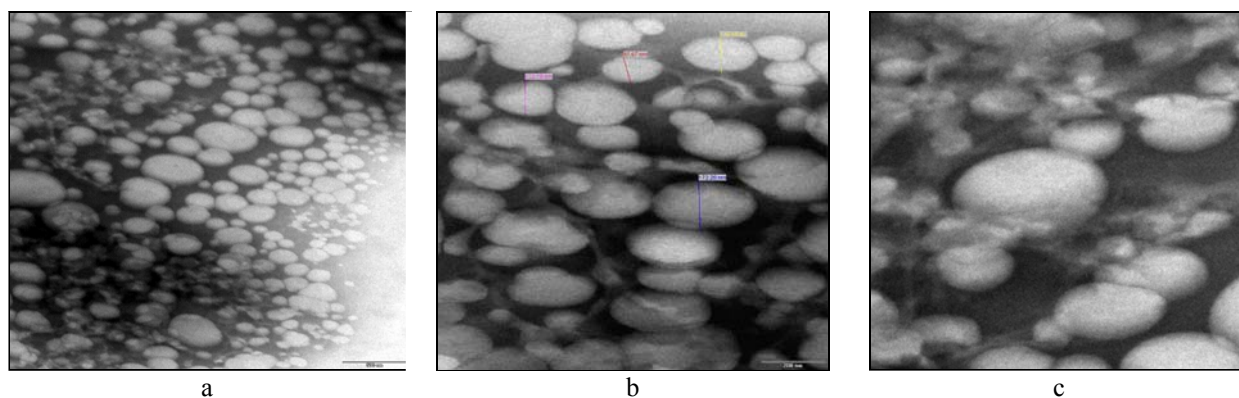
Fig. 4 illustrates the TEM micrographs, which demonstrated that the droplets were spherical in shape and measured less than 200 nm. In addition, it is apparent from all these Figures that Carb 940 formed a well-recognized network. This network most likely confirmed the increase in the intrinsic viscosity of the samples prepared containing this polymer. TEM micrographs also confirmed the nano-meter size of emulsions droplets that was previously determined by photon correlation technique, which appeared to be relatively comparable. However, with the two techniques employed in our experiments, minor differences in the measured droplets were considered likely since the techniques used bore totally different principle of measurement. TEM depends on droplets diffraction, while photon correlation spectroscopy (Malvern) is based on droplets projection.

**Table 5:** Calculated intrinsic viscosity of nano-scaled emulsions containing different concentrations of Carb 940 using TEA solutions as the external phase at various pH values.

Formulation external phase pH using TEA	$\eta_i^a$ (Poise) Carb 940 0.50% w/w	$\eta_i^a$ (Poise) Carb 940 0.75% w/w	$\eta_i^a$ (Poise) Carb 940 1.00% w/w
<b>6.5</b>	47.39 ± 3.50	4503.70 ± 29.41	17100.59 ± 538.60
<b>7.0</b>	69.09 ± 2.16	6235.21 ± 335.51	31826.15 ± 598.15
<b>7.4</b>	148.00 ± 7.36	7565.41 ± 160.89	51589.81 ± 751.58
<b>8.0</b>	208.26 ± 8.59	12052.24 ± 411.07	76772.95 ± 5137.63

<sup>a</sup> Calculated intrinsic viscosity. (Mean ± SD, n = 3)

All formulations contain ibuprofen: TEA solution pH 7.4: POEs: Tween 80: sodium benzoate at a ratio of 5: 35.08: 23.70: 36.02: 0.20. Reference calculated intrinsic viscosity equals to 122.98 ± 2.44 (Poise).

**Fig. 4:** Droplets size measurement and structural image of formulation 11 obtained by TEM.

#### *In vitro permeation and in vivo pharmacodynamics studies*

Later, *in vitro* release studies through full-thickness Wistar rat skin was performed in which the drug concentrations in all formulations evaluated were determined via the HPLC method developed by our research group (Abdullah *et al.*, 2010). The release profile has shown that the usage of Carb 940 at the selected concentration resulted in a significant decrease in ibuprofen penetrating ability compared to Carb-free formula as a result of the gel-network formation that can decrease the drug thermodynamic energy (Abdullah *et al.*, 2011a).

However, ibuprofen release-profile from formula 11 was statistically comparable to that of the reference. Consequently, two types of terpenes (menthol and limonene) were employed as permeation enhancers. The addition of these enhancers at 10% w/w concentration has drastically improved ibuprofen permeation through full thickness rat skin with enhancement ratios of 1.47 and 1.37, respectively. Furthermore, the *in vivo* pharmacodynamic activity of the drug was also improved as a result of the enhanced permeation profile (Abdullah *et al.*, 2011a). Upon the completion of all necessary formulation, *in vitro* and *in vivo* evaluation procedures, the stability of the optimized formulae was also assessed. This study has shown that the optimized formulae were

stable in terms of droplets size, conductivity, pH, flow properties and drug content (Abdullah *et al.*, 2011b)

#### DISCUSSION

The measured zeta potential was not changed upon the addition of various types and concentrations of Carbopols<sup>®</sup> because of the usage of sodium hydroxide and potassium dihydrogen phosphate for the production of phosphate buffer of specific pH value. These electrolytes can neutralize the carboxylate groups present on the polymer backbone. This neutralization process is necessary to accomplish the gel-forming and viscosity or rheology-modifying properties of these polymers. The ionized carboxyl groups repel each other via electrostatic repulsion, a process which adds to the rigidity of the formed network (Lubrizol Advanced Materials Inc. TDS-237, 2009). Indeed, ions of the buffer present in an equilibrium situation between diffusion into the external medium and attraction by the carboxylate anions (Piau, 2007). Therefore, it is expected for such a system to show a constant zeta potential with only minor unperceived changes.

The change in the formulae appearance and droplets size is explained on the basis of gel-forming and viscosity-increasing capabilities of all members of the Carbopol<sup>®</sup> polymers family. Given that the droplets size is

unswervingly considered as the most influential factor in dispersions appearance, the molecular weight and structure of these polymers were huge enough to cause such a noticeable change in the size of the dispersed droplets measured by photon correlation technique. Therefore, the selected rheology modifier was further examined using TEM to determine its structure, network and size. Observation over a period of three months did not reveal any sign of emulsion instability, like upward creaming or downward sedimentation, since Carbopol® resins dramatically increased the viscosity of all formulations prepared. Hence, the latter effect adds to the dispersion stability as stated by Stock's Law of sedimentation (Attwood, 2004; Sinko, 2006). In general, the enlargement of droplets is attributed to the fact that Carbopol® resins are capable of forming a gel network when they come in contact with aqueous media (Lu and Jun, 1998). This gelling capability rises upon the addition of alkaline neutralizing entities (Piau, 2007). Carb U10, as mentioned earlier, is a hybrid polymer between Carb 934 and Carb 940, with superior swelling properties and application domains. Therefore, at this level of study and the use of PB pH 7.4 as the external phase, it was found that the size of the droplets of the nano-scaled emulsions increased from about 20 nm to more than 160 nm. This increment in droplets size is related to the type and concentration of the polymer used.

The significant increase in the measured intrinsic viscosity of formulations 4, 5 and 6 comprising different concentrations of Carb 940 compared to formulae 7, 8 and 9 containing Carb U10 at the same concentration can be explained on the basis that Carb 940 dispersion had the highest viscosity amongst the other types of Carbopols® used in this study (Lubrizon Advanced Materials Inc. CP-26, 2000). In addition, pH 7.4, the pH value that was used for the preparation of the polymer dispersion lies in the range of 6-8. This pH range is described by "Lubrizon" to be the most suitable range to produce the powerful thickening properties of this polymer (Lubrizon Advanced Materials Inc. Pharmaceutical Bulletin (PB) 6, 2008). These results revealed that all types of Carbopols® are capable of improving the flow properties. However, this improvement is highly related to the type and concentration of the polymer used. Moreover, formulae 6 and 9 containing 1.00% w/w of Carb 940 and Carb U10 respectively showed a comparable intrinsic viscosity to that of the reference. However, the droplets size of these formulations was significantly higher than that of the basic formula, where the highest droplets size was disclosed by formula 9 as shown in Table 3. Therefore, Carb 940 was selected for further studies to assess the effects of another neutralizing compound, namely TEA, on its flow controlling properties.

As aforementioned, such organic amine can produce a better neutralizing activity of the carboxylate groups present on the surface of such polymers. TEA, as an

organic amine, has wide applications in the production of cosmetic preparations, pharmaceutical ointments, textile and household cleaning products (Kraeling *et al.*, 2004). Higher neutralization leads to a chain of events including: higher electrostatic repulsions, better gelling characteristics and more rigid structures. All these measures can lead to an advanced improvement in the flow properties of emulsions prepared. However, this may be accompanied by an additional increase in the droplets size of the dispersed phase globules.

This change can be explained based on the fact that Carbopol® resins are highly sensitive when they react to the type and pH of neutralizing solution used in the preparation of their aqueous dispersions. Alternatively, higher pH value means higher concentration of basic entities. This effect measurably leads to more efficient ionization of Carbopols® carboxylate groups. As a result, huger and stiffer dispersion is formed which clearly explains the results obtained.

## CONCLUSIONS

From this study, it can be concluded that out of all types of Carbopols® used neutralized by PB pH 7.4, Carb 940 was found to be the best in modifying the rheological properties of nano-scaled emulsion designed for topical application. Drastic increase in droplets size was apparent with increasing the polymers concentrations.

Neutralizing Carb 940 by TEA (an organic amine) made this polymer stronger as a gelling agent and as a rheology modifier, since the calculated intrinsic viscosity of the sample studied dramatically raised and the droplets size simultaneously showed increase. The highest concentration of TEA used led to a significant enlargement in droplets size of samples containing this polymer.

Formula 11 compared to the reference showed a significantly higher intrinsic viscosity with a droplets size of less than 200 nm, which is ideal for a nano-scaled emulsion. These results were confirmed by TEM that showed a well formed Carb 940 gel-network, which can be held responsible for the improvement in the flow properties and the increase in droplets size.

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