

Chapter Three

Emulsions stability of some *Acacia* gums and blends

3.1. Introduction

3.1.1. Emulsion definition

The term emulsion is derived from the word emulgeo meaning “to milk”. Milk is example of a natural emulsion. “An emulsion is a heterogeneous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, whose diameter, in general, exceeds 0.1 μ m. Such systems possess a minimal stability, which may be accentuated by such additives as surface-active agents, finely divided solids, etc.” Friberg, Mandell, and Larsson (1969) elucidated the significance of liquid crystalline phases on emulsion stability. This was reflected in the IUPAC (1972) definition of an emulsion: “in an emulsion, liquid droplets and/or liquid crystals are dispersed in a liquid.” Sharma and Shah (1985) defined both micro- and macroemulsions, differentiating them on the basis of size and stability. Macroemulsions were defined as “mixtures of two immiscible liquids, one of them being dispersed in the form of fine droplets with (a) diameter greater than 0.1 μ m in the other liquid. Such systems are turbid, milky in color and thermodynamically unstable.” Microemulsions were defined as “clear thermodynamically stable dispersions of two immiscible liquids.” The dispersed range consists of small droplets in the range of 100–1000 Å . Food

macroemulsions are unstable systems, even with the addition of emulsifiers. Emulsifiers are added to increase product stability and attain an acceptable shelf-life. The function of an emulsifier is to join together oily and aqueous phases of an emulsion in a homogeneous and stable preparation (Waginaire, 1997). The main characteristic of an emulsifier is that it contains in its molecule two parts. The first part has a hydrophilic affinity, while the second has a lipophilic affinity. Emulsifier selection is based upon final product characteristics, emulsion preparation methodology, the amount of emulsifier added, the chemical and physical characteristics of each phase, and the presence of other functional components in the emulsion. Food emulsifiers have a wide range of functions. The most obvious is to assist stabilization and formation of emulsions by the reduction of surface tension at the oil–water interface, to alter the functional properties of other food components and third function is to modify the crystallization of fat.

3.1.2. Classification of emulsions

Emulsions classified according to the nature of the emulsifier or the structure of the system. A number of different terms are, commonly, used to describe different types of emulsions and it is important to classify these terms (Mason, Wilking, Meleson, Chang, and Graves, 2006; McClements, 2010; Tadros, Izquierdo, Esquena, and Solans, 2004). The range of droplets size for each type of emulsion is quite arbitrary and is defined in terms of the physical and thermodynamic

properties of emulsions. A conventional emulsion, also known as a macroemulsion, typically, has a mean droplet diameter (MDD) between 100nm and 100 μ m. Macroemulsions are the most common form of emulsions used in the food industry and are found in a variety of products, including milk, beverages, mayonnaises dips, sauces and desserts. Macroemulsions are prone to physical instability (e.g.: gravitational separation, flocculation and coalescence). Especially when exposed to environmental stresses (Aoki, Decker, and McClements, 2005; Djordjevic, Cercaci, Alamed, McClements, and Decker, 2007; Given, 2009; Klinkesorn, Sophanodora, Chinachoti, Decker, and McClements, 2005). Nanoemulsions are dispersions of nanoscale droplets with a MDD between 20-100nm. It is worth nothing that in old days, "microemulsion" was also used to refer to emulsions comprised of submicron droplets (Tang, Sudol, Silebi, and El-Aasser, 1991). In contrast to nanoemulsions, miniemulsions have droplets in the range from 100nm to 1 μ m. Because miniemulsions and macroemulsions are, thermodynamically, unstable, microemulsion is a, thermodynamically, stable system and is formed, spontaneously, with droplet size between 5 to 50nm (McClements, 2010). Nanoemulsions are the same as microemulsions since they both typically contain oil, water and surfactant and also have similar MDD.

3.1.3. Food hydrocolloids

One of the key functional roles of food hydrocolloids is in the preparation of emulsions and in the control of emulsion shelf-life. Product applications include carbonated soft drinks (Tan, Friberg, Larsson, and Sjöblom, 2004), ice-cream (Goff, 1997), and sauces and dressings (Sikora, Badrie, Deisingh, and Kowalski, 2008). Most hydrocolloids can act as stabilizers (stabilizing agents) of oil-in-water emulsions, but only a few can act as emulsifiers (emulsifying agents). The latter functionality requires substantial surface activity at the oil–water interface, and hence the ability to facilitate the formation and stabilization of fine droplets during and after emulsification (Dickinson, 2003, 2004). The most, widely, used polysaccharide emulsifiers in food applications are gum arabic (*Acacia senegal* var. *senegal*), modified starches, modified celluloses, pectin, and some Galactomannans (Dickinson, 2003; Garti and Reichman, 1993). The surface activity of these hydrocolloids has its molecular origin in either (i) the non-polar character of chemical groups attached to the hydrophilic polysaccharide backbone (in hydrophobically modified starch/cellulose) or (ii) the presence of a protein component linked covalently or physically to the polysaccharide (some gums, pectins, etc.). Protein ingredients derived from milk and eggs are the most commonly used food emulsifying agents; but these are not hydrocolloids (Dickinson, 1992). Due to its unique hydrophilic character, gelatin is, really, the

only protein that can be properly categorized as a hydrocolloid. Gelatin does have some emulsifying ability, but its more characteristic roles are as a colloid stabilizer and gelling agent(Dickinson, 2009).

3.1.4. Natural gums

Natural gums are obtained as exudates from different tree species, which exhibit unique and diverse physicochemical properties and have a wide variety of applications (Verbeken et al., 2003). Commercially important tree gums include gum arabic, gum karaya and gum tragacanth (Phillips and Williams, 2001). Earlier, natural plant exudate gums were classified into various groups depending on the basic chain structure and structural units that contribute to the polymeric form of the gum (Vinod et al., 2008).

The exudate gum from *Acacia senegal* var. *senegal* (gum hashab) is the most abundant and available, and is also the most common commercial gum arabic. The second, most available gum is from *Acacia seyal* var. *seyal* locally known as gum talha(Al-Assaf et al., 2005).

3.1.5. Emulsion formation

The first step in the formation of a stable emulsion is dispersion of one liquid phase in another liquid phase. A critical factor in that emulsification process is the formation of a monomolecular layer at the lipid/water interphase. During emulsion formation there is a large increase in surface area (up to several thousand fold),

which is dependent upon the number and size of the droplets. To form and disperse these droplets, a substantial amount of energy or work must be supplied. Since emulsifiers reduce the surface tension, the addition of emulsifiers reduces the amount of work that must be done to form the emulsion. The most common method of emulsion formation is the application of mechanical energy via vigorous agitation. The emulsifier is first dissolved in the aqueous or organic phase depending on the solubility of the emulsifier and on the type of emulsion desired. Next, sufficient agitation to cause surface deformation and large droplet formation is applied during the addition of one phase to the other. The next step is the disruption of droplets. To form a stable emulsion and prevent coalescence, sufficient amount of emulsifier must be available to adsorb at the aqueous/organic interphase. The emulsifier lowers the Laplace pressure, which facilitate droplets deformation and disruption (Walstra, 1983). After droplets formation, the emulsifier partitions into the interphase of the aqueous/organic system stabilize the emulsion. Droplet size, which is, directly, related to the emulsification procedure, depends on the amount of emulsifier added, the type of emulsifier, and the emulsification temperature. There are several possible methods for emulsion formation and a wide range of equipment is available for emulsion formation. These methods include shaking, stirring, and injection, and the use of colloid mills, homogenizers, and ultrasonics.

Emulsions have a long history of use and are, widely, used in many products encountered in everyday life. The importance of emulsions in food industries justifies a great deal of basic research to understand the origin of instability and methods to prevent their break down (Adheeb Usaid, Premkumar, and Ranganathan, 2014).

3.1.6. Beverage emulsions

The term “beverage emulsion” is used to describe a group of products that have similar composition, preparation and physicochemical properties, for example, fruit drinks, punches and sodas. In soft drinks, the beverage emulsion may provide flavor, color and cloudy appearance for the beverage, or just the cloudiness. In fact, beverage emulsions are either flavor emulsions to provide the beverage with flavor, cloudiness and color as in certain formulae or cloud emulsions to provide only cloudiness. Beverage emulsions are oil-in-water (O/W) emulsions that are prepared in a concentrated form and then diluted several hundred times in acidic sugar solution prior to consumption in order to produce the finished beverage, either carbonated or non-carbonated . Several studies have been mainly carried out on physical stability and rheological properties of the beverage emulsion (Buffo and Reineccius, 2002; Mirhosseini, Tan, Hamid, and Yusof, 2008; Taherian, Fustier, and Ramaswamy, 2006). *Acacia* gums are widely used to form beverage emulsions, which are unique in that they are consumed in a, highly, diluted form.

They are prepared as an emulsion concentrate, which is later diluted several hundred times in acidic sugar solution (Mirhosseini et al., 2008). Emulsions should be stable, to significant degree, in both concentrate and dilute form (Buffo and Reineccius, 2002). While substantial work has been done on studying emulsions and factors that influence their stability, the process of making emulsions is still treated as an art rather than a science. Product and legal constraints put severe limits on materials that can be used to insure emulsion stability, particularly, the introduction of weighting agents into the oil phase. Thus, stability of beverage emulsions is a chronic problem that plagues the flavor and beverage industries.

3.1.7. Oil-in-Water Emulsion Definition

An emulsion is composed of two immiscible liquids (usually oil and water), with one of the liquids being dispersed as small spherical droplets in the other liquid (McClements, 2005). In foods, the diameter of the droplets usually lies between 0.1 μm to 100 μm (Dickinson, 1992; Friberg and Larsson, 1997). Emulsions can be classified based on the relative spatial distribution of the oil and water phases. Oil-in-Water (O/W) emulsion refers to oil droplets dispersed in an aqueous phase, such as milk, cream, dressings, beverage, dips and sauces. Water-in-Oil (W/O) emulsion refers to water droplets dispersed in an oil phase, such as margarine and butter. The material that makes up the droplets is called dispersed, discontinuous, or internal phase, whereas the material that makes up the surrounding liquid is called

dispersing, continuous or external phase. It is also possible to create various types of multiple emulsions, such as oil-in-water-in-oil (O/W/O), water-in-oil-in-water (W/O/W), oil-in-water-in-water (O/W/W) (Kim, Decker, and McClements, 2006).

3.1.8. Basic Preparation Method

The process of converting bulk oil and bulk water into an emulsion, or of reducing the size of the droplets in a preexisting emulsion, is known as homogenization. It can be carried out by using the homogenizer, such as a high shear mixer, a high pressure valve homogenizer, a colloid mill or an ultrasonic homogenizer, to apply intense mechanical agitation to a liquid mixture (McClements, 2005).

Conventional emulsions are, inherently, thermodynamically unstable systems because the contact between oil droplets and water molecules is unfavorable, and they are intended to breakdown over time (Dickinson, 1992; Friberg, Larsson, and Sjoblom, 2004). In this case, in order to prepare emulsions that are kinetically stable over time (a few days, weeks, months or years), emulsifiers and stabilizers are required. An emulsifier is a surface-active substance that adsorbs to the surface of emulsion droplets to form a protective coating that prevents the droplets from aggregating with one another, but they do have different emulsifying mechanisms. Gum *Acacia*, an amphiphilic that is a mixture of anionic polysaccharides and protein fraction, tends to stabilize emulsions primarily through steric interactions (Castellani, Al-Assaf, Axelos, Phillips, and Anton, 2010; Dickinson, 2003; Garti

and Leser, 2001; Wilde, 2000). Octenyl succinic anhydride (OSA) modified starch, is composed of starch molecules that have been chemically reacted with Octenyl succinic anhydride to give some hydrophobic character (Given, 2009; Tan et al., 2004). Whey protein isolate is a mixture of amphoteric globular protein and polysaccharides, and tends to stabilize emulsions primarily through electrostatic interactions (Chanamai and McClements, 2002; Wilde, 2000).

3.1.9. Emulsion Preparation Parameters and Conditions

For freshly, prepared concentrated emulsion, formulation composition and homogenization conditions are two main aspects determining emulsion quality (McClements, 2005). Formulations are composed of oil, preservative, deionized water, gum emulsifier, and/or weighting agent, especially the ratio of oil to emulsifier is critical in the formulation. Homogenization conditions include homogenization time, pressure and numbers of passes. For further diluted beverage emulsion, besides formulation composition and homogenization conditions, pH is also a key factor, and citric acid is often used to adjust final pH (McClements, 2005).

3.1.10. Emulsion Instability Mechanisms

Emulsion stability describes the ability of an emulsion to resist changes in physicochemical properties over time (McClements, 2005). It is always important to; clearly, identify the dominant/major physicochemical mechanisms responsible

for the instability of a particular emulsion before, effective, strategies can be developed to improve emulsion stability. Figure (3.1) shows instability mechanisms. A variety of different, physicochemical, mechanisms are involved in emulsion instability performance, including gravitational separation, flocculation, coalescence, Ostwald ripening and phase inversion (McClements, 2007). Gravitational separation is the process whereby droplets move upward or downward. When droplets have a lower density than the surrounding liquids, they move upward, which is called creaming. When droplets have a higher density than the surrounding liquids, they move downwards, which is called sedimentation. Flocculation and coalescence are two types of droplet aggregation. Flocculation occurs when two or more droplets stick together to form an aggregate in which the droplets retain their individual integrity, whereas coalescence is the process when two or more droplets merge together to form a single larger droplet (McClements, 2005). Ostwald ripening results from mass transport of dispersed phase material through the continuous phase, larger droplets grow at the expense of smaller droplets. Phase inversion is the process whereby an O/W emulsion changes into a W/O emulsion, or vice versa (McClements, 2007).

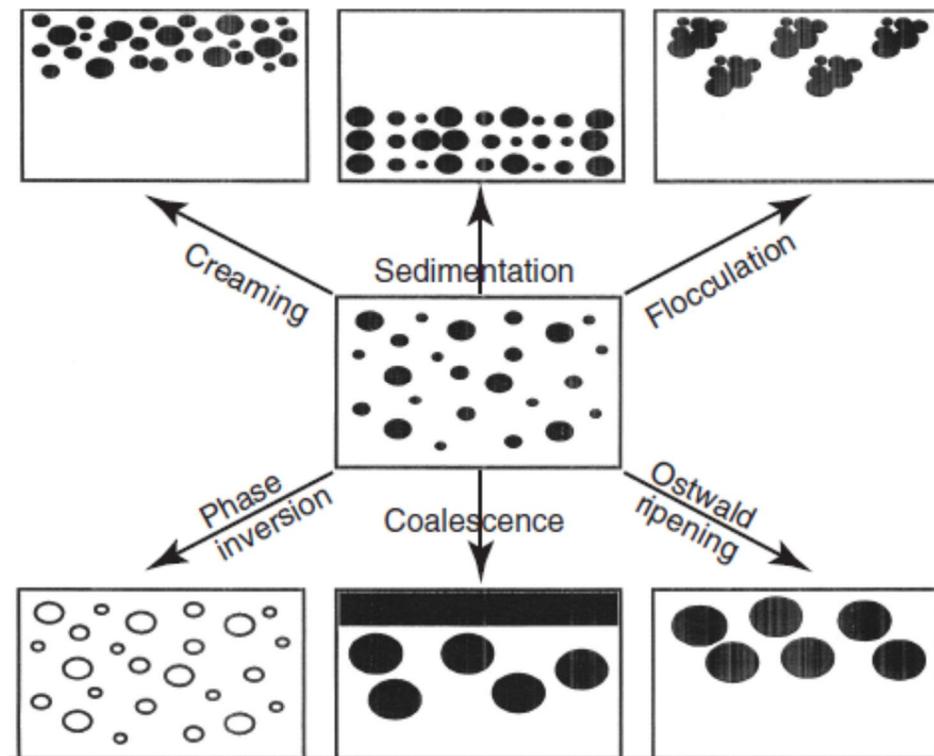


Figure (3.1): Schematic representation of the various breakdown processes in emulsions.

3.1.11. Factors Influencing Emulsion Properties

Emulsion properties are, strongly, influenced by droplets characteristics, including their concentration, particle size, droplet charge, interfacial properties and colloidal interactions (Dickinson, 1992; Friberg et al., 2004; McClements, 2005, 2007; Qian, Decker, Xiao, and McClements, 2011).

Droplet concentration in an emulsion plays an important role in determining its structure, stability, appearance and quality (McClements, 2005). Therefore, it is important to be able to clearly specify its droplet concentration. Droplet concentration is, usually, characterized in terms of dispersed phase volume fraction or dispersed phase mass fraction. Many of the most important properties of emulsion-based food products are determined by droplet size, such as stability (gravitational separation, flocculation, coalescence, etc.), optical properties (lightness or turbidity), and rheology (viscosity or interfacial tension). Therefore, it is particularly important to be able to reliably measure droplet size, which can be reported as a radius or a diameter (McClements, 2007). Emulsions can be classified as “monodisperse” and “polydisperse”. When, all, droplets have the same size, the emulsion is referred as monodisperse. For a polydisperse emulsion, it is characterized as particle size distribution (PSD), in which the particle concentration is usually presented as either volume percent or number percent of droplets within a particular class (McClements, 2005). The particle size analyzer

provides a full PS of an emulsion, which contains information about the size characteristics of, all, particles presents, also providing insight into the possible origin and nature of any, instability, changes in the emulsion (McClements, 2007). The two, most commonly, used mean particle size values are the surface-weighted mean diameter and the volume-weighted mean diameter. In general, the volume-weighted mean diameter is more sensitive to the presence of large particles (McClements, 2005).

The charge on a droplet is important because it determines the nature of its interactions with other charged species or its behavior in the presence of electrical fields (McClements, 2005). In many food emulsions, droplets are prevented from aggregation by ionic emulsifiers that adsorb to their surface and prevent them from coming close enough because of electrostatic repulsion (Dickinson, 1992). The properties of emulsions stabilized by ionized emulsifiers are very sensitive to pH and ionic strength of the aqueous phase. The electrical characteristics of a droplet are, often, characterized in terms of its surface electrical potential, surface charge density or ζ -potential. The ζ -potential is defined as the distance away from the droplet surface below which the counter ions remain, firmly, attached to the droplet. Among all the charge parameters, ζ -potential is much easier to measure and it accounts for the adsorption of any charged counter ions, therefore ζ -potential is broader used than others (Hunter, 1986).

The droplet interface consists of a narrow region that surrounds each emulsion droplet, which contains a mixture of oil, water and surface-active molecules (Hunter, 1986). It can have an appreciable impact on many of the most important bulk physicochemical properties of food emulsions (McClements, 2005). Therefore, it is of importance to elucidate the factors that determine the composition, structure, thickness, rheology and charge of interfacial region (McClements, 2007). Colloidal interactions control whether emulsion droplets aggregate or remain as separate entities as well as the characteristics of any aggregates formed. There are many different kinds of colloidal interactions that may act in food emulsions, including van der Waals, electrostatic, steric, depletion, hydration and hydrophobic interactions (McClements, 2005). These individual interactions vary in (attractive or repulsive) sign, (weak to strong) magnitude, and (short to long) range. Generally, droplets tend to aggregate when attractive interactions dominate, but remain as individual entities when repulsive interactions dominate (Dong, 2012; McClements, 2005). Surface-active hydrocolloids may act as emulsifiers and emulsion stabilizers through adsorption of protective layers at oil-water interfaces, and interactions of hydrocolloids with emulsion droplets may affect stability with respect to aggregation, e.g., gum arabic, Dickinson (2003) studied and summarized the main factors affecting emulsion

stability and pointed out that for a biopolymer to be more effective in stabilizing emulsion droplet, it should exhibit four characteristics:

- I. Strong adsorption, implying that the amphiphilic polymer has a substantial degree of hydrophobic character to keep it permanently anchored to the interface to lower interfacial tension dramatically.
- II. Fully surface coverage, implying that there is sufficient polymer present with the ability to completely saturate the surface.
- III. A thick steric stabilizing layer is formed, implying that the polymer is hydrophilic with high molecular weight and high solubility.
- IV. A charged stabilizing layer is formed if the polymer is not sufficiently thick.

For a freshly prepared concentrated emulsion, the most obvious initial manifestation of instability is creaming, which leads on to macroscopic phase separation, then into two separated regions of cream and serum by clarification (Dickinson, 2003, 2009).

The objectives of this work are:

- To prepare, monitor, and evaluate stability of emulsions formed using gums under study (*Acacia senegal* var. *senegal* (ASG), *Acacia mellifera* (AMF), *Acacia seyal* var. *seyal* (ASY), and *Acacia tortilis* var. *raddiana* (ATR)).

- To prepare, monitor, and evaluate emulsions of gum blends using different proportion of gums; ASG and AMF gums from *Vulgares* series, and ASY and ATR gums from *Gummiferae* series.
- To study the viability of using gum blends in emulsion preparation.

3.2. Materials and Methods

3.2.1. Materials

Gum powder of ASG, AMF, ASY, and ATR. Isopropyl myristate (IPM) oil from Spectrum Chemical MFG. CORP., 90.0% assay and 0.846-0.854g/ml specific gravity at 25°C, was used as the dispersed phase. Deionized water (18.2 MΩ cm⁻¹; resistivity) was obtained from Barnstead NANO pure® Diamond TM ultrapure water system was used throughout this work.

3.2.2. Preparation of gums emulsions

30% (W/W) gum solutions were prepared using magnetic stirrer for 3h and left overnight for full hydration and then centrifuged using (VELOCITY 18R REFRIGERATED CENTRIFUGE, Dynamica) for 10 min. at 2500 rpm to remove insoluble particles and air bubbles. The solution was filtered using 100 μm mesh (Sanchez, Renard, Robert, Schmitt, and Lefebvre, 2002). 6.67g of 30% (w/w) gum solution was weighed in glass vial, and appropriate amounts of IPM oil were added to form 20%, 17.5%, 15%, and 10% oil concentration. Deionized water was added to make a total of 10g. Emulsion was prepared using (ULTRA TURRAX

T25 basic IKA) homogenizer at 22,000 rpm for 5min. IKA S25N-8G, Ident No: 1024200, was used as dispersing tool. Emulsions formed were kept in incubator of 45.0±0.1C subjected to accelerating aging process. Stability of emulsions was determined by observing emulsion's physical changes for period of 28 days.

3.2.3. Preparation of emulsions of gum blends

Emulsion was prepared using various combinations of gums with IPM oil, to achieve 20% total gum content and 20% oil for a total of 10 g. Gums of ASG and AMF were mixed 1:1(w/w). For ASY and ATR gum blend emulsions were prepared using formula as shown in Table 3.1.

Table3.1. The content of components used in emulsion of gum blend of ASY and ATR (W/W %).

Sample	Proportions of 20% gum blend		IPM Oil%	Water%
	ASY %	ATR%		
100% ATR	00	100	20	60
1:4 ASY:ATR	20	80	20	60
3:7 ASY:ATR	30	70	20	60
2:3 ASY:ATR	40	60	20	60
1:1 ASY:ATR	50	50	20	60
4:1 ASY:ATR	80	20	20	60
100% ASY	100	00	20	60

3.2.4. Morphology of Emulsion

Light polarizing microscope from Leica model PM RXP by Leica Microsystems GmbH, Germany was used to observe the emulsion droplets formed. Polarizing microscope unit was equipped with high voltage beam, polarizing unit and a JVC Color Video camera with model KY F550, interfaced with personal computer with Leica QWin image analysis software. Leica QWin software is a powerful tool in digital images capturing with capability of measuring and analysis of droplet size of sample. Samples were examined under the microscope with previous dilution in deionized water (three times w/w). A drop of emulsion was deposited on a microscope slide and then covered with a cover slip (Desplanques, Renou, Grisel, and Malhiac, 2012). Observation of changes in droplets size and appearance of emulsion of the *Acacia* gums under the study has been made for one and seven days of incubation at 45C.

3.2.5. Particle Size Analysis

The average particle size of *Acacia* gum emulsions were determined using (COULTER N4 PLUS), 0.05g of emulsions were diluted with 5ml deionized water. A 1cm path length clear quartz cuvette was used for particle size measurements at 25C.

3.2.6. Phase Separation Stability Test

Destabilization of *Acacia* gums and blends emulsions in terms of phase separation was observed by measuring heights of phases and calculating emulsion fraction as percentage (Krstonošić, Dokić, Dokić, and Dapčević, 2009; Radi and Amiri, 2013), while incubating the emulsions for four weeks at 45C.

3.2.7. Dye Solubility Test

This test was conducted to determine the continuous phase of the emulsions and identification of emulsion system whether O/W or W/O system. Two types of dyes were used in this test; soluble only in oil phase which is Sudan IV and a water soluble dye which is Crystal violet.

3.3. Results and Discussion

3.3.1. Emulsion Morphology

The morphological structures of the four *Acacia* gum species emulsions were studied over a period of seven days of incubation at 45C and different IPM oil concentrations (Figures 3.2-3.3 and 3.5-3.6). The oil droplets were spherical in shape and retain their structure in spite of dense emulsion reflecting a high degree of stability and elasticity of droplets exhibited by each gum. *Acacia* gums increase the viscosity of the continuous phase and form a network around the droplets. Figures (3.2 and 3.3) show the morphology of ASG and AMF gum emulsions; AMF gum formed smaller droplets than ASG gum. Figure (3.4); show the

morphology of the emulsion formed by a blend of ASG and AMF gums (1:1); and it was observed that the droplets formed were smaller than that formed by the parent gum.

For ASY and ATR gum emulsions; figures (3.5 and 3.6) there is a clear growth of the oil droplet size for ASY, but ATR gum formed uniform, stable, and smaller droplets. Emulsions formed with different proportion of ATR and ASY gum blends (Figure 3.7 and 3.8) show smaller and more stable droplets than that formed by each gum alone. Generally, the higher proportion of ATR gum in the blend the more uniform and smaller oil droplets formed. The best combination ratio is 3:7 of ASY and ATR gum blend.

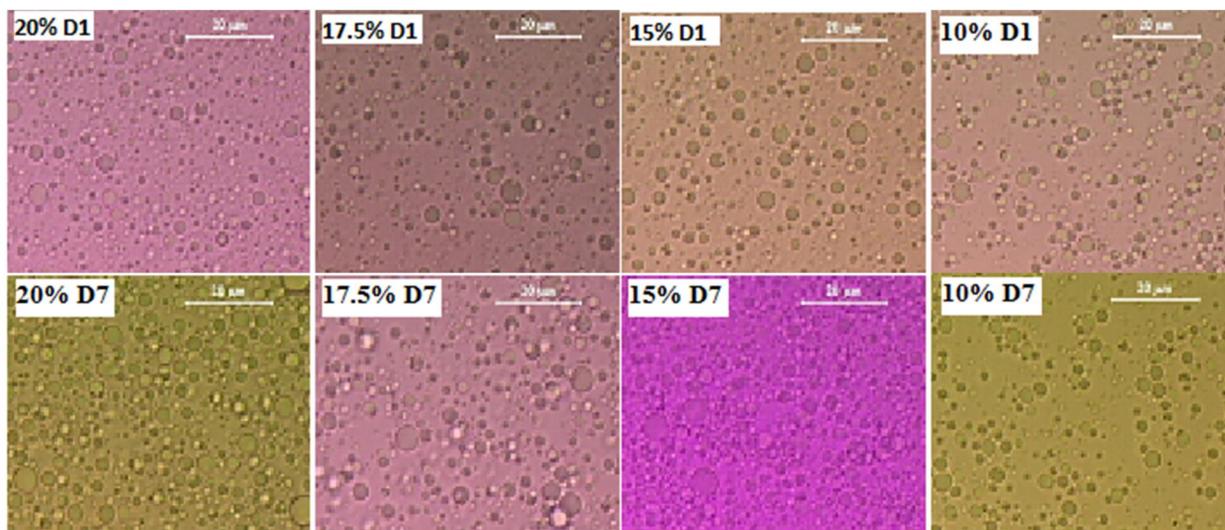


Figure (3.2): Micrographs of ASG gum emulsion samples with different IPM oil concentrations; 20%, 17.5%, 15%, and 10%; after one day (D1) and seven days (D7) of incubation at 45C.

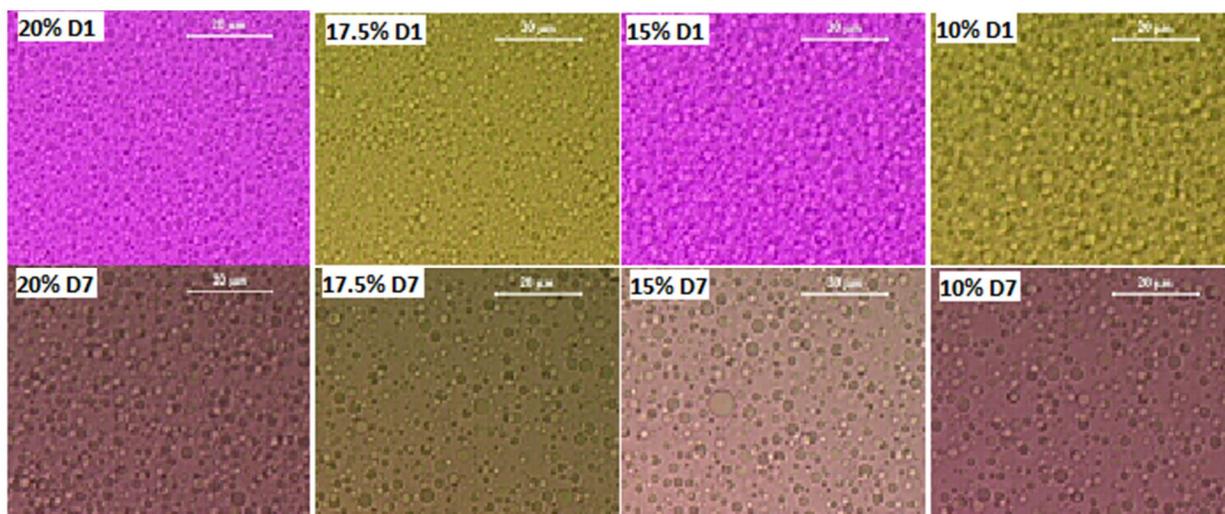


Figure (3.3): Micrographs of AMF gum emulsion samples with different IPM oil concentrations; 20%, 17.5%, 15%, and 10%; after one day (D1) and seven days (D7) of incubation at 45C.

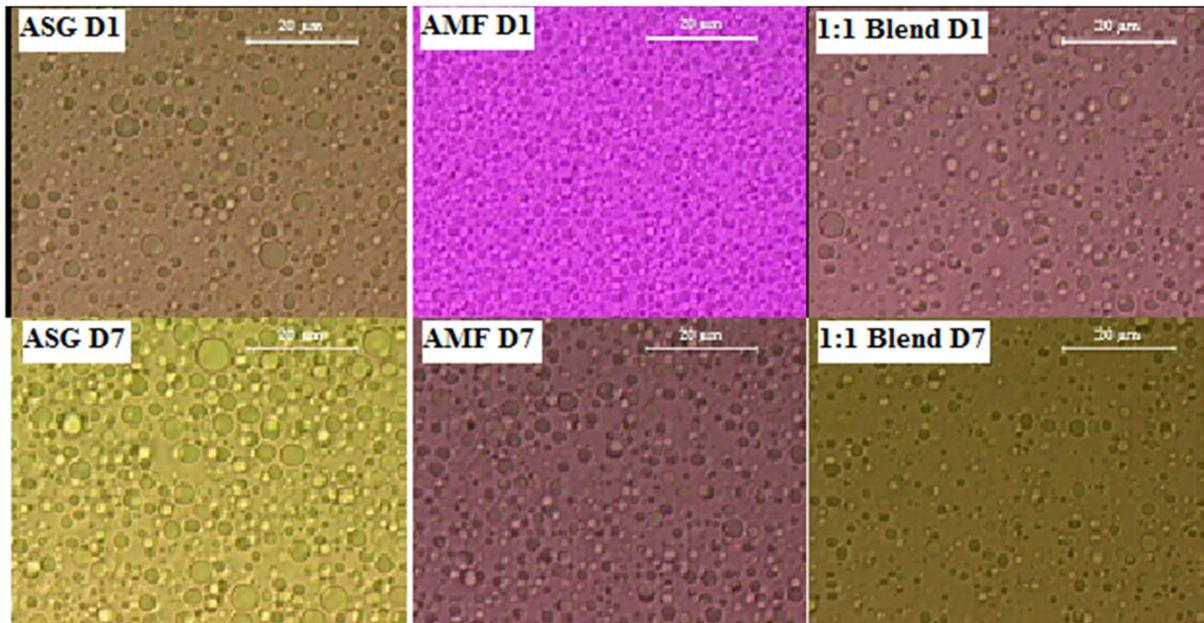


Figure (3.4): Micrographs of ASG, AMF, and 1:1 gum blend emulsion samples with 20% IPM oil concentrations after one day (D1) and seven days (D7) of incubation at 45C.

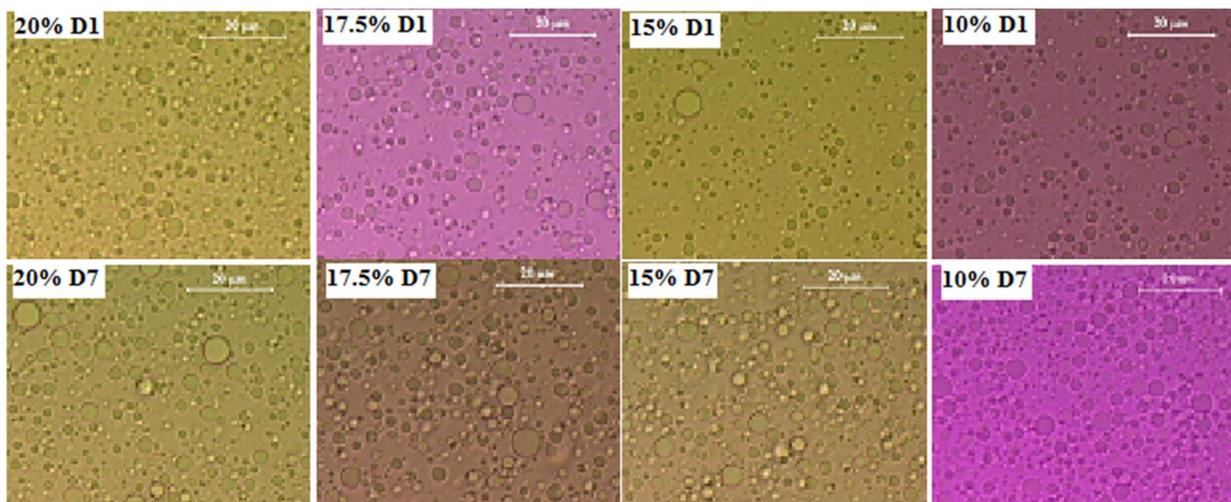


Figure (3.5): Micrographs of ASY gum emulsion samples with different IPM oil concentrations; 20%, 17.5%, 15%, and 10%; after one day (D1) and seven days (D7) of incubation at 45C.

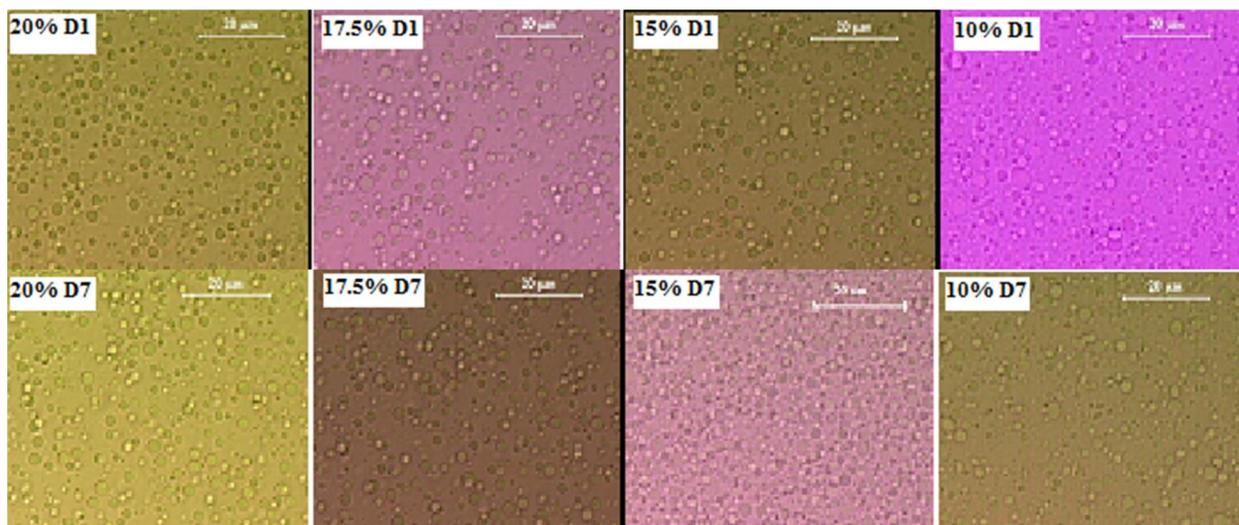


Figure (3.6): Micrographs of ATR gum emulsion samples with different IPM oil concentrations; 20%, 17.5%, 15%, and 10%; after one day (D1) and seven days (D7) of incubation at 45C.

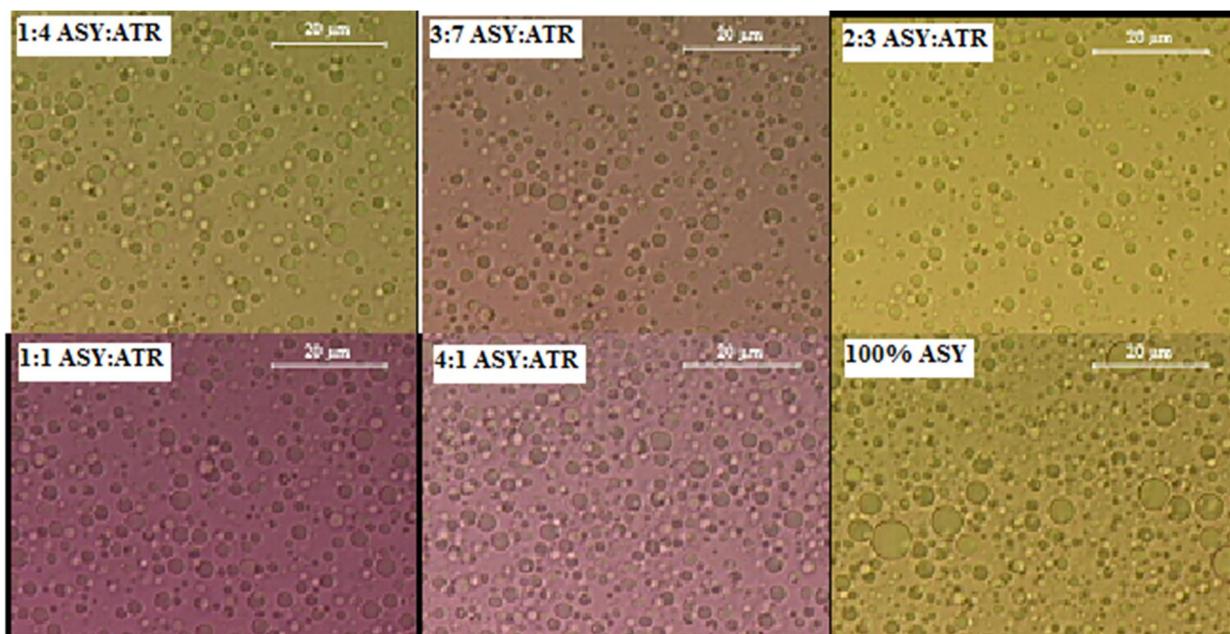


Figure (3.7): Micrographs of ASY and ATR gum blends emulsions (1:4, 3:7, 2:3, 1:1, and 4:1 ASY: ATR) with 20% IPM oil concentration after one day of incubation at 45C.

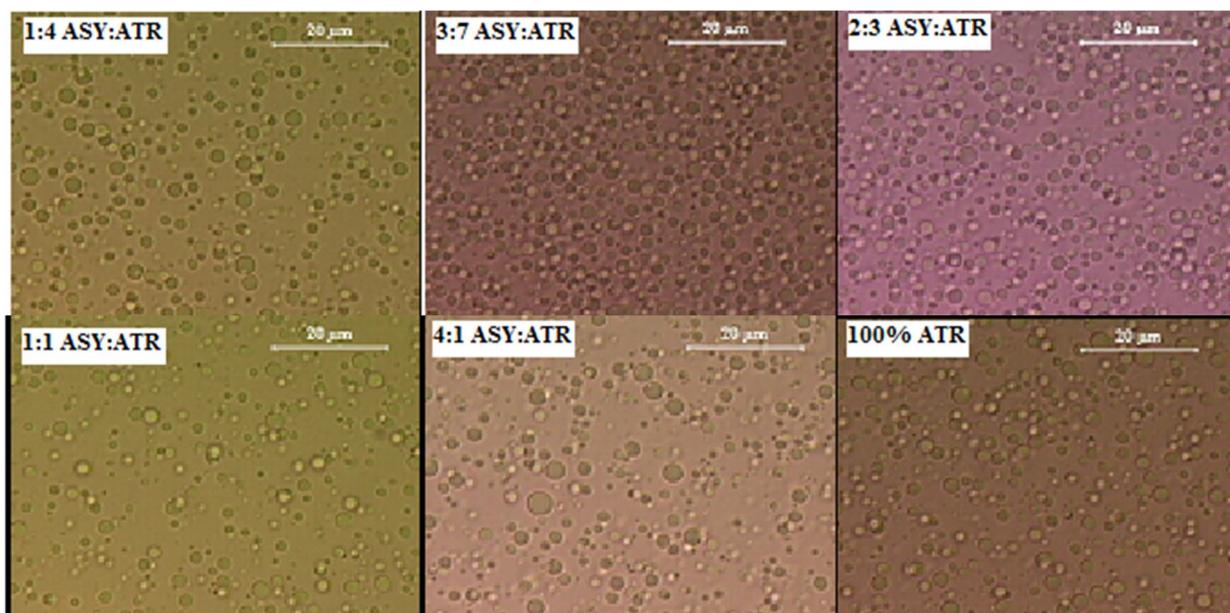


Figure (3.8): Micrographs of ASY and ATR gum blends emulsions (1:4, 3:7, 2:3, 1:1, and 4:1 ASY: ATR) with 20% IPM oil concentration after seven days of incubation at 45C.

3.3.2. Particle size analysis

Acacia gum emulsions were analyzed with COULTER N4 PLUS to measure droplet size. Average droplets size were an average of three measurements. Data of droplets size measurements were tabulated (Tables 3.2-3.5). Figures (3.9-3.12) show the average droplets size for each gum emulsion using different IPM oil concentrations, and Figure (3.13) shows the average droplet size of the different *Acacia* gums emulsions at 20% IPM oil concentration. All emulsions were subjected to accelerated test on incubation at 45C.

From Figure (3.13) ASG gum emulsion exhibits the largest average droplet size and ATR gum emulsion shows the smallest average droplet size that is droplet size increases in the order ATR, ASY, AM and ASG gum emulsion. These results might be attributed to the high protein content (10.38%) of ATR gum (Abdelrahman, 2011). This assumption was not applicable for the other gum samples, from AMF (Mohamed Bilal, 2010) and ASG (Osman, 1993) which contain higher protein contents than ASY gum with 1.61% protein content (Hassan, 2000).

Table 3.2. Average droplets size of ASG gum emulsion.

Number of Days	Average Droplets Size (nm)			
	20% IPM	17.5% IPM	15% IPM	10% IPM
Day1	2397.1	2974.3	2997.9	2798.1
Day3	2425.4	2835.3	2799.9	2711.2
Day7	2952.4	2814.6	2819.5	2960.4
Day14	3020.6	2403.2	2666.3	3086.7
Day21	3078.6	2867.2	2970	2996.7
Day28	3071.1	3119.2	3313.3	3132.5

Table 3.3. Average droplets size of AMF gum emulsion.

Number of Days	Average Droplets Size (nm)			
	20% IPM	17.5% IPM	15% IPM	10% IPM
Day1	2524.0	2314.6	2738.4	2581.8
Day3	2586.3	2874.9	3051.8	2626.3
Day7	2790.5	2800.9	3213.6	2739.6
Day14	2802.5	2928.5	3375.2	2987.6
Day21	2814.5	2986.2	3097.3	3076.1
Day28	2841.8	2996.5	3190.6	3096.9

Table 3.4. Average droplets size of ASY gum emulsion.

Number of Days	Average Droplets Size (nm)			
	20% IPM	17.5% IPM	15% IPM	10% IPM
Day1	2151.2	2189.8	1960.6	1963.8
Day3	2380.5	2339.9	2324.0	2112.4
Day7	2455.6	2610.1	2057.1	2454.6
Day14	2554.2	2394.6	2539.8	2388.6
Day21	2827.5	2923.2	2464.7	2130.6
Day28	2372.6	2441.7	2339.5	1749.0

Table 3.5. Average droplets size of ATR gum emulsion.

Number of Days	Average Droplets Size (nm)			
	20% IPM	17.5% IPM	15% IPM	10% IPM
Day1	1706.7	1705.6	1804.6	1676.0
Day3	1740.4	1760.2	1877.6	1761.3
Day7	1687.0	1797.0	1788.6	1611.0
Day14	1590.1	1662.3	1796.0	1607.7
Day21	2035.1	2227.7	2225.6	2011.3
Day28	2151.0	2160.6	1972.1	1902.7

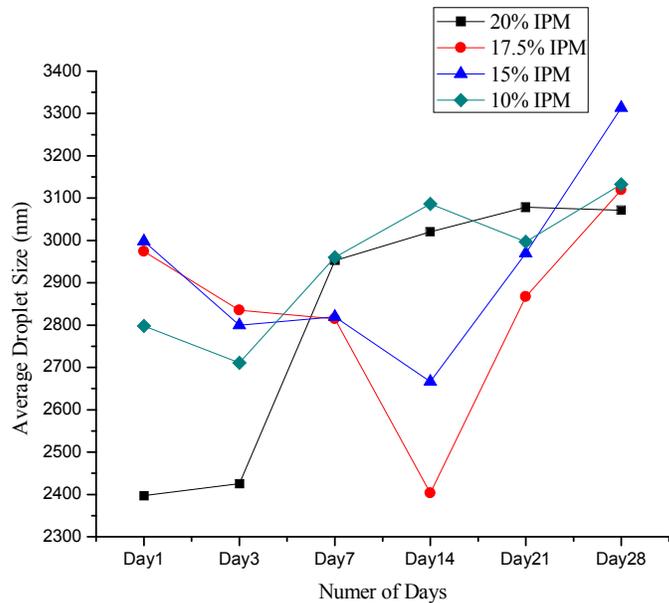


Figure (3.9): Variation of average droplet size of ASG gum emulsion with time at various IPM concentrations.

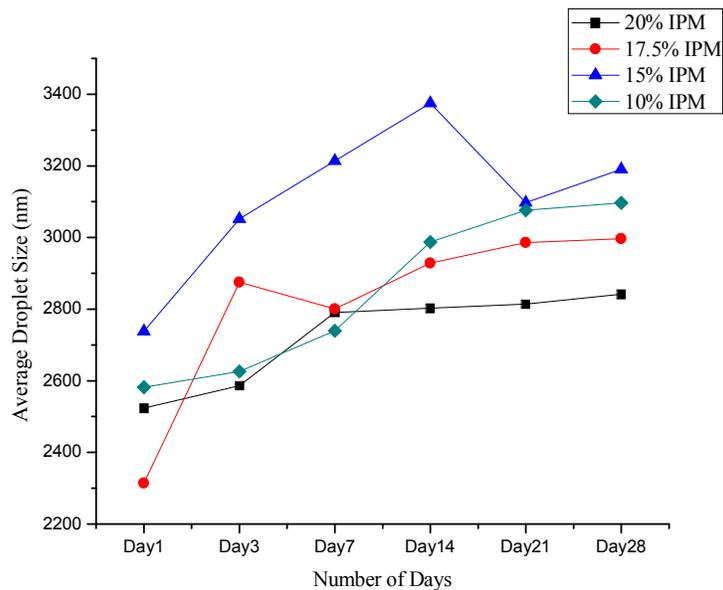


Figure (3.10): Variation of average droplet size of AMF gum emulsion with time at various IPM concentrations.

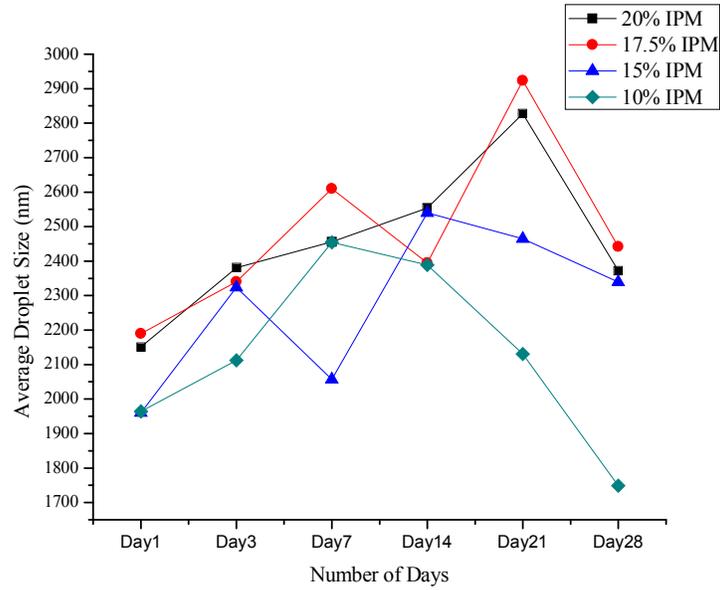


Figure (3.11): Variation of average droplet size of ASY gum emulsion with time at various IPM concentrations.

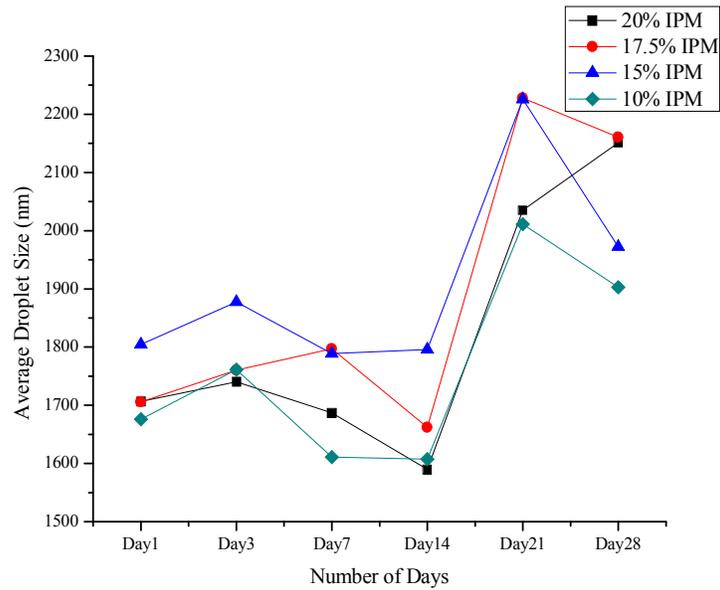


Figure (3.12): Variation of average droplet size of ATR gum emulsion with time at various IPM concentrations.

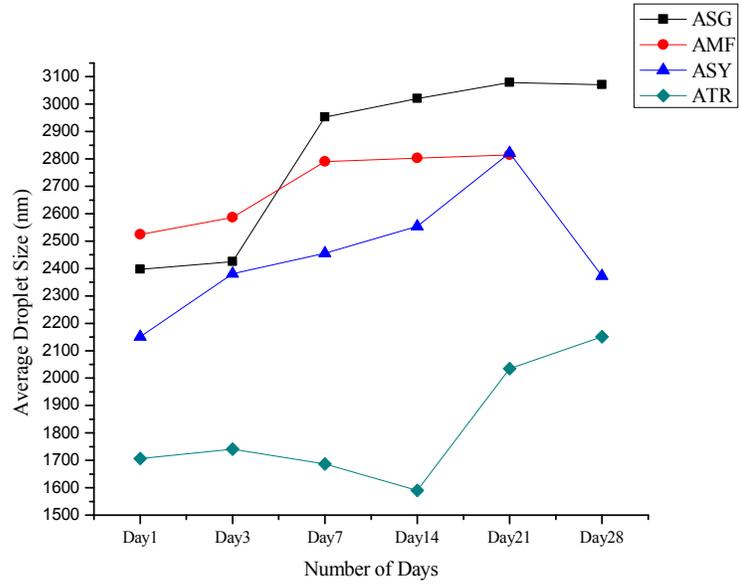


Figure (3.13): Variation of average droplet size of ASG, AMF, ASY and ATR gum emulsions with time at 20% IPM concentration.

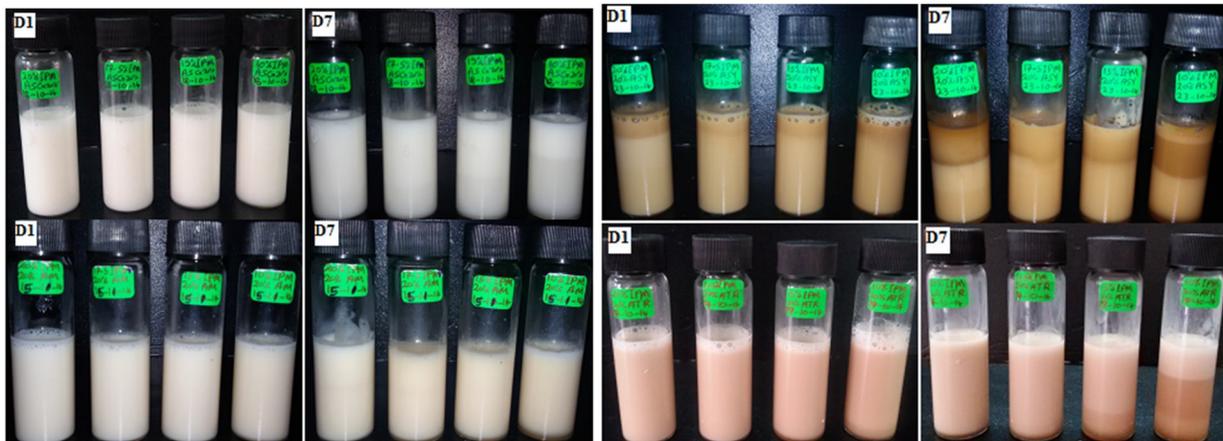


Figure (3.14): ASG, AMF, ASY, and ATR gums emulsions at day 1 (D1) and day7 (D7).

3.3.3. Phase Separation Stability Test

Emulsion fractions were measured for *Acacia* gum emulsions and their blends incubated at 45C over 28 days (Figures 3.15-3.20). It was observed that AMF gum emulsion was more stable against phase separation than ASG gum emulsion. ASY gum emulsion was stable against phase separation up to the seventh day for the 20% IPM oil concentration where lower concentrations of IPM start to separate gradually. On the other hand ATR gum emulsion was more stable compared to ASY, and the order of stability against phase separation was; ATR>ASY>AMF > ASG. These findings were supported by the previous results and the 20% oil concentration emulsion was the best concentration for all gums as emulsions with lower oil concentrations were less stable. Gum blends emulsions were prepared with 20% IPM oil concentration as it was the concentration of the best emulsion stability. Figure (3.19) shows the emulsion fraction percentage for ASG, AMF, and 1:1 blend proportion; which was more stable against phase separation than the emulsions of each gum alone. Figure (3.20) shows the emulsion fraction percentage of ASY, ATR gums and their blends with different proportions (1:4, 3:7, 2:3, 1:1, and 4:1); it was observed that generally, the more ATR gum proportion the more stable the emulsion against separation, but emulsion formed with 30% ASY and 70% ATR, (3:7), gum combination was the most stable.

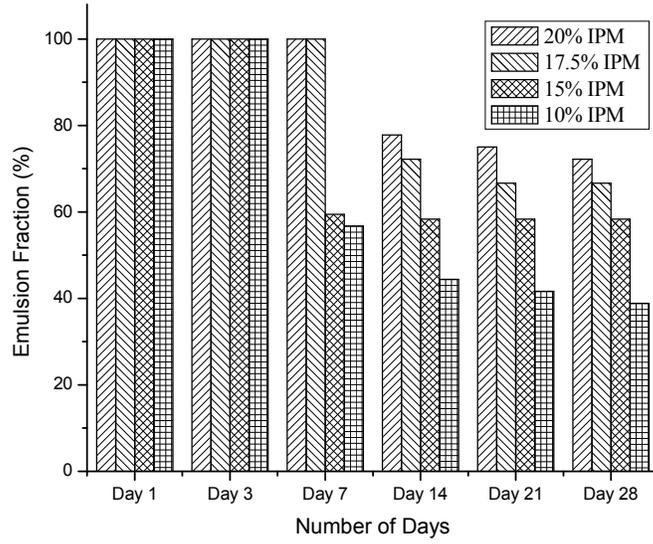


Figure (3.15): ASG Gum Emulsion Fraction%.

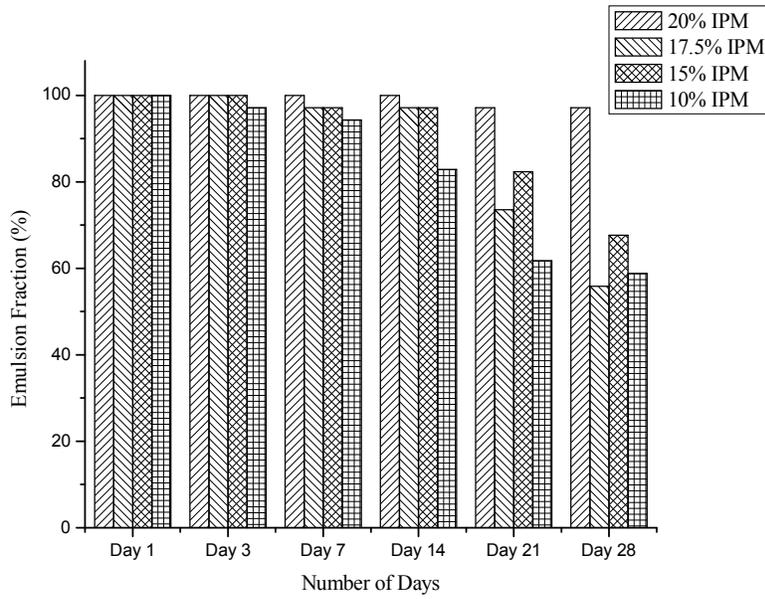


Figure (3.16): AMF Gum Emulsion Fraction%.

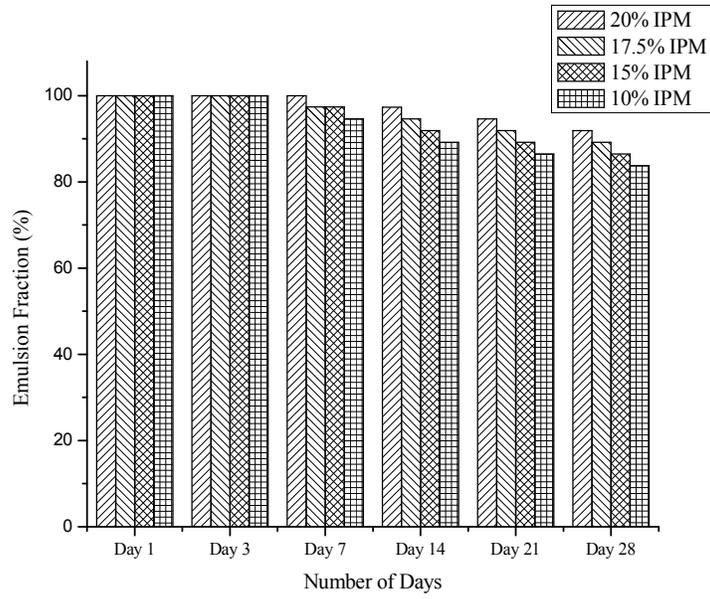


Figure (3.17): ASY Gum Emulsion Fraction %.

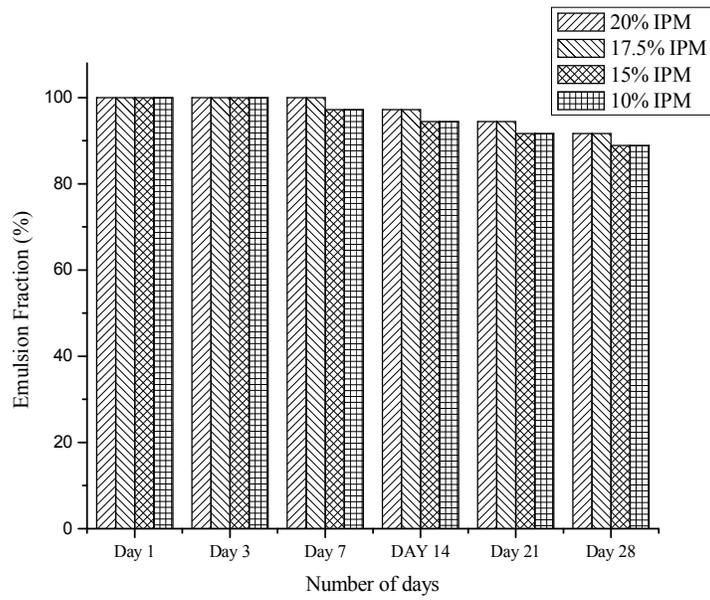


Figure (3.18): ATR Gum Emulsion Fraction %.

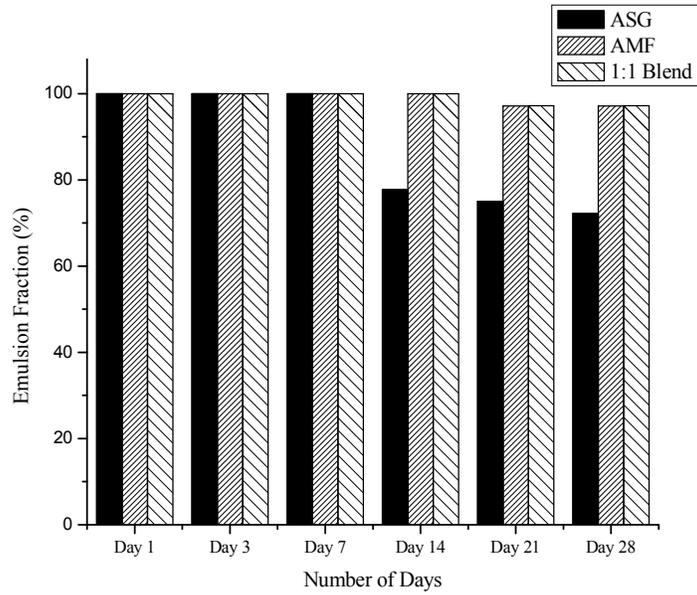


Figure (3.19): Emulsion fraction% of ASG, AMF, and 1:1 gum blend with 20% IPM oil concentration incubated at 45C for 28 days.

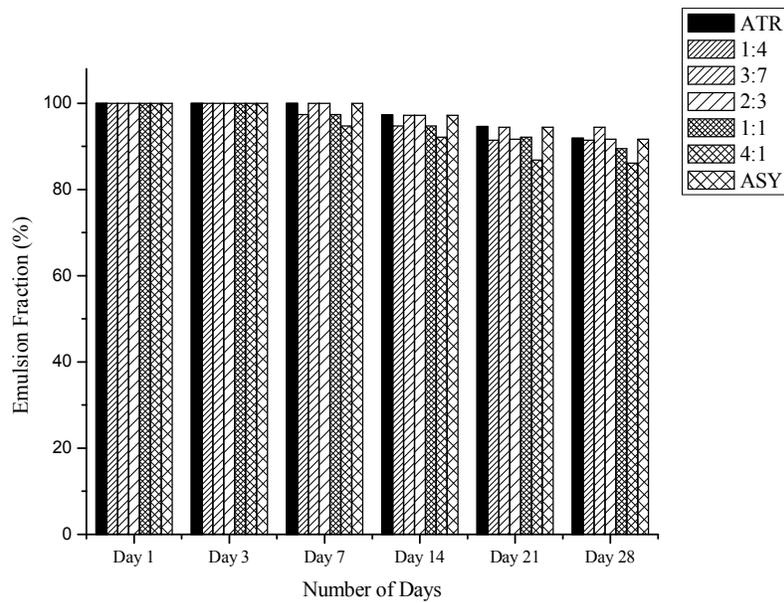


Figure (3.20): Emulsion fraction % of ATR and ASY gums and blends of 1:4, 3:7, 2:3, 1:1 and 4:1; proportions with 20% IPM oil concentration for 28 days of incubation.

3.3.4. Dye solubility test

Sudan IV and crystal violet were added to the emulsion system of ASG, AMF, ASY and ATR gums in three different ways; I. dissolved (few crystals of Sudan IV in IPM oil and of crystal violet in water), II. solid, without stirring and, III. solid, with stirring. Results show that dissolved Sudan IV dye does not spread as does the solid dye with and without stirring suggesting that the dispersed phase of the emulsion is not an oil base (Samanta, Ojha, and Mukherjee, 2010) (Figure 3.21). While crystal violet solution and solid, with and without stirring, spreads in emulsions implying that the dye can be dispersed into the continuous phase suggesting that the dispersing medium was water (Figure 3.22). Figure (3.23) shows some of the gum blends emulsions with Sudan IV and crystal violet dyes. These observations confirmed that ASG, AMF, ASY and ATR gum emulsions and their blends are O/W emulsion systems.

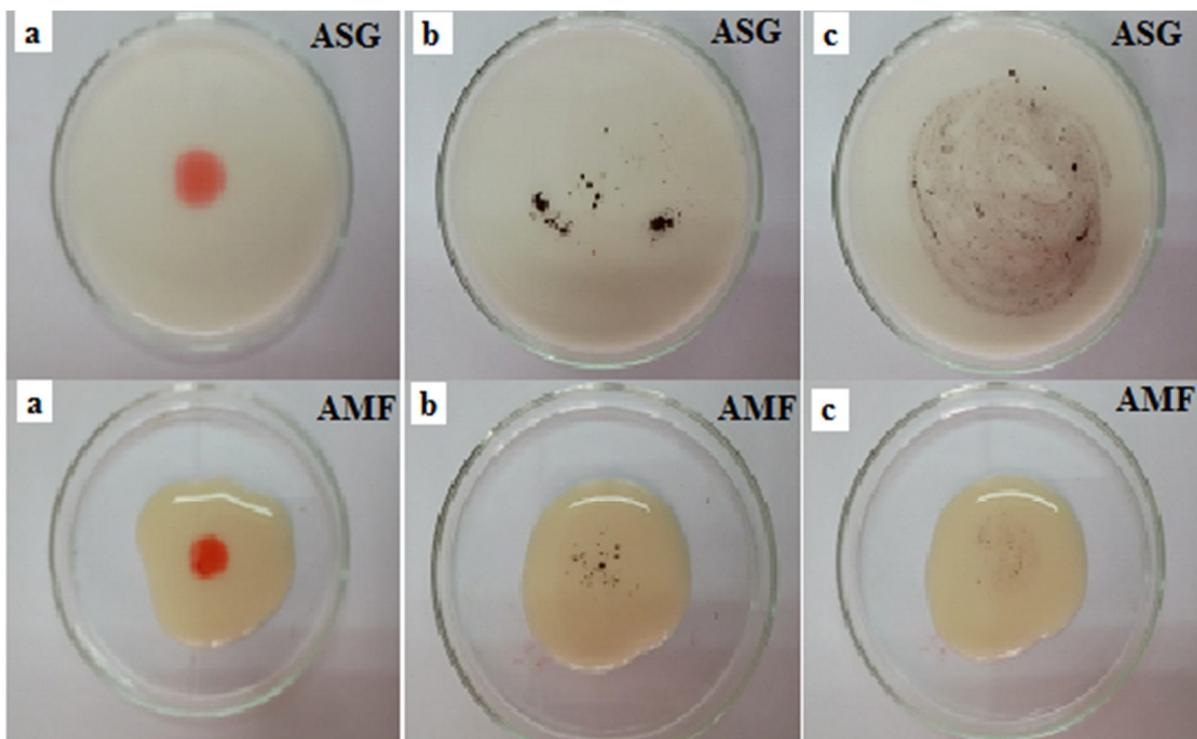


Figure (3.21): ASG and AMF gum emulsions and Sudan IV; (a) dissolved, (b) solid, and (c) stirred solid.

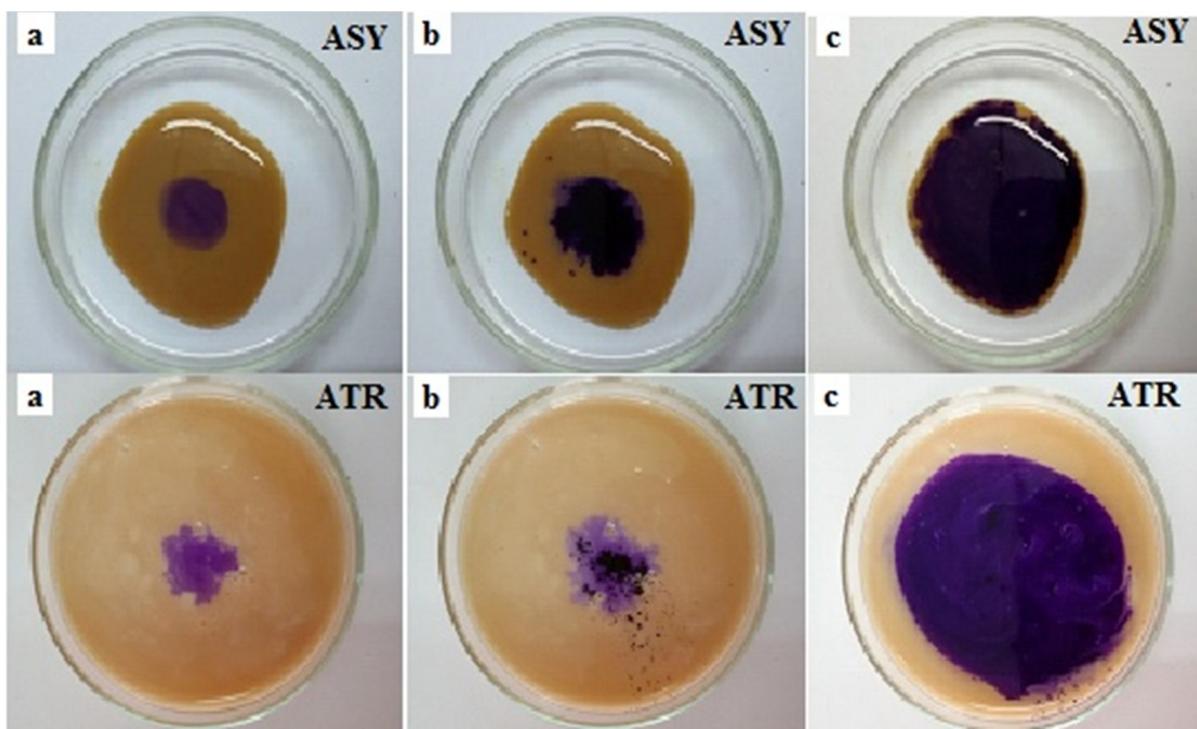


Figure (3.22): ASY and ATR gum emulsions and Crystal violet; (a) dissolved, (b) solid, and (c) stirred solid.

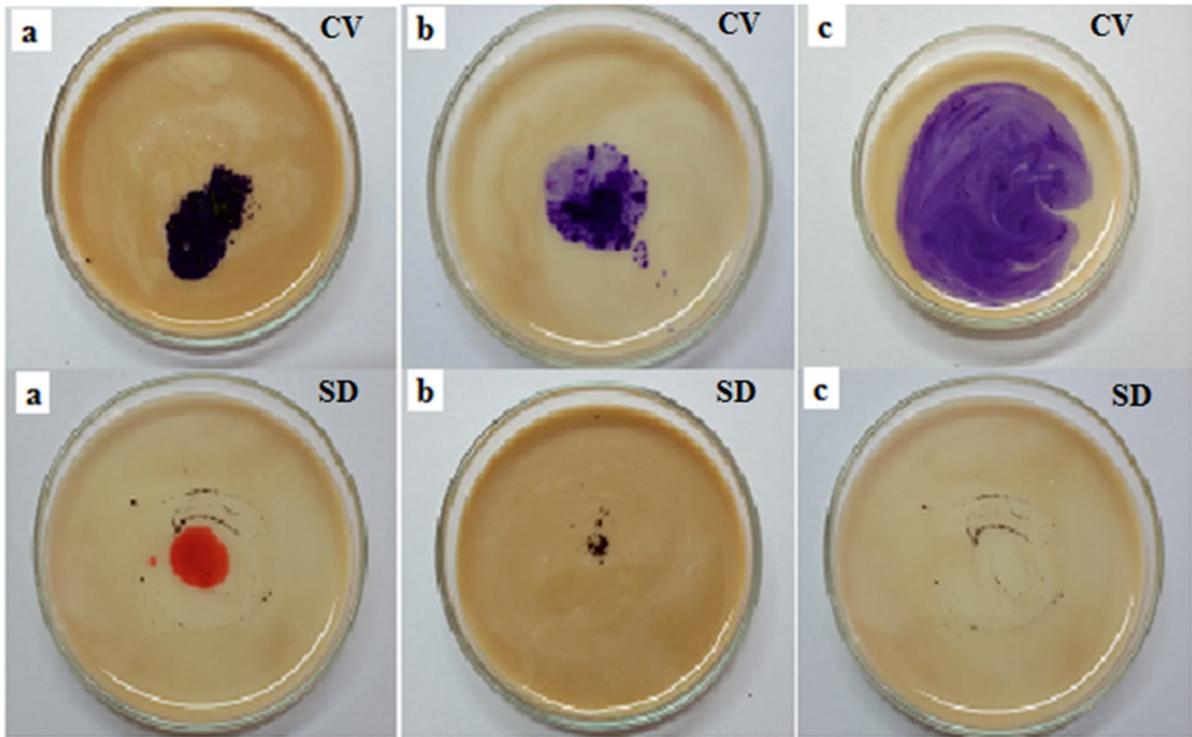


Figure (3.23): ASY and ATR gum blend emulsions with Crystal violet (CV) and Sudan IV (SD); (a) dissolved, (b) solid, and (c) stirred solid.

3.4. Conclusions

The emulsions of *Acacia* gums under study formed at different IPM oil concentrations (20%-10%) showed;

- Variable degree of stability; with ATR gum emulsion is the most stable one followed by ASY, AMF, and ASG, and the optimum formulation was the 20% oil concentration.
- When gums from the same series (*Vulgares* and *Gummiferae*), were blended at the optimum formula, they formed emulsions with remarkable stability.