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Multiple Emulsions: An Overview of Formulation, Characterization, Stability and Applications

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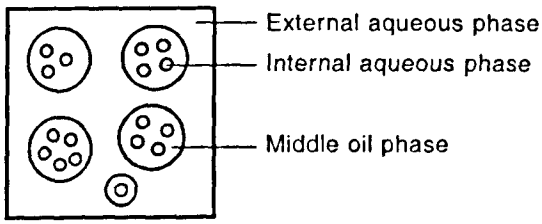
Multiple emulsions are a type of polydisperse, systems where both oil-in-water and water-in-oil emulsions exist simultaneously. Multiple emulsions have been proposed to have numerous uses including their use as prolonged drug delivery system. The inherent instability of these systems needs to be overcome before they find potential application in pharmaceuticals. This review focuses on formulation, preparation, characterization, causes of instability, stabilization, and potential applications of multiple emulsions.

Multiple emulsions are polydispersed systems where the dispersed phase contains the droplets of the continuous phase. These double emulsions are of two types: w/o/w type multiple emulsions and o/w/o type multiple emulsions. In the w/o/w type multiple emulsions (fig. 1), small water droplets are dispersed in bigger oil droplets and these oil droplets are again dispersed in a continuous aqueous phase. Similarly in o/w/o type multiple emulsions (fig. 1), small oil droplets are dispersed in larger aqueous droplet and these aqueous droplets are again dispersed in a continuous oil phase.

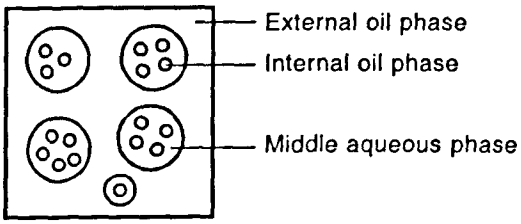
The basic rationale for the use of w/o/w and o/w/o type multiple emulsions as a means of prolonged delivery of drugs is that the drug contained in the innermost phase is forced to partition itself through several phases prior to release at the absorption site. Thus the partition and diffusion coefficient of the drug and the strength of the middle membrane phase, which is a multimolecular layer of oil, water, and emulsifier molecules at both the interfaces of multiple emulsion system, controls the drug release from these systems.

Preparation and yield of multiple emulsions:

A two step procedure developed by Matsumoto *et al.*,[1] is the most common method for preparation of multiple emulsions. For preparing a w/o/w emulsion, a simple w/o emulsion is first prepared by gradual addition of internal aqueous phase to oil phase (containing a suitable lipophilic emulsifier) with continuous stirring which is then added to the external aqueous phase (containing a suitable hydrophilic emulsifier) with continuous stirring. Suitable modifications, like a pre-emulsification step[2], can be made in this process to achieve proper emulsification. Similarly for preparing o/w/o emulsion, an o/w emulsion is prepared first which is then emulsified with external oil phase.



w/o/w multiple emulsion



o/w/o multiple emulsion

Fig. 1: W/o/w type and o/w/o type of multiple emulsions.

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Sphere-in-oil-in-water emulsions are specialised forms of multiple emulsions where microspheres containing the drug form the innermost phase[3-5]. Such emulsions are made by emulsifying (preformed) microspheres with an oil phase to produce a sphere-in-oil emulsion which is again emulsified with an external aqueous phase to produce s/o/w emulsion[4,5]. Partial phase solublisation inversion technology is another technique for one step production of stable w/o/w emulsions[6]. This process is based on controlled salting out of the hydrophilic emulsifier during the multiple droplet formation. This process is reported to assure a droplet multiplicity of more than 85%.

Yield (or entrapment efficiency) can be expressed in two ways; first is the percentage of multiple droplets relative to simple droplets and second as the fraction of internal aqueous phase entrapped as multiple droplet[7]. Primary phase volume ratio, secondary emulsification time, mixing speed, secondary phase volume ratio and additives affect the yield of multiple emulsion.

Yield of a w/o/w emulsion decreases with an increase in the primary phase volume ratio[7] but Matsuomoto *et al.*,[1] found no significant effect of internal phase volume on yield. Collings[8] has suggested the optimal aqueous phase volume to be 25-50 percent of the oil phase. Primary phase volume ratio also has an effect on particle size and viscosity of the w/o/w emulsion and it was found that the entrapment efficiency increased from 81.2% to 99.3% with change in phase ratio from 1:1:2 to 1:4:5[2]. Increase in primary volume of inner aqueous phase increases the cumulative drug release from w/o/w emulsions[9,10]. Second emulsification step is critical as excess of shearing can cause rupture of multiple droplets leading to an emulsion with marked simple o/w character and decreased yield[1,7,11,12]. Davis and Walker[7] found that yields of w/o/w emulsions decreased in a zero order manner as a function of increasing second emulsification time. An increase in secondary emulsification time reduced particle size and increases amount of drug in external aqueous phase thus decreasing the yield[8]. Increase in second sonication time might retard the phase separation but increases the release rate from these systems and droplets size decreased with increase in second emulsification time[13]. Davis and Walker[7] reported yield to be relatively independent of the secondary phase volume ratio. In contrast Matsumoto *et al.*[1] found that the yield of liquid paraffin based emulsion system increased from 55% to 90% when secondary phase volume ratio was increased from 0.1 to 0.5.

Yield/stability of a w/o/w multiple emulsion increases upon addition of various agents viz. Surfactants and sorbitol[14], sodium chloride[14,15] dextrose[16], bovine serum albumin[15], glucose[17] to the internal aqueous phase of w/o/w emulsions. This improvement in yield in case of surfactants was found to be due to a decrease in the interfacial tension between the internal aqueous phase and oil phase of the w/o/w emulsion. This decrease in interfacial tension leads to formation of smectic type liquid crystals upon addition of hydrophilic surfactant to the internal aqueous phase[14]. Osmotic additives (sodium chloride, bovine serum albumin and dextrose) when added to the internal aqueous phase cause the transfer of water from external aqueous phase to internal aqueous phase leading to improvement in formation percentage and stability due to increase in the viscosity of the emulsion[15-17]. Increase in the concentration of glucose or sodium chloride in aqueous inner phase increases the percentage of solute entrapped in the inner aqueous phase and viscosity of the emulsion and later delayed the separation of aqueous phase and release of the solute[18].

Formulation of multiple emulsions:

The oils used to prepare multiple emulsions include liquid paraffin, vegetable oils such as sesame oil, olive oil, arachis oil, isopropyl myristate and others. Mixtures of oils can also be used to minimize the differences in specific gravity between the oil and aqueous phase of emulsion[2] and to vary the viscosity of the oil phase in order to control the movement of solute across the oil membrane[19]. In case of o/w/o emulsion system, the two oil phases can be same or different[20-22]. A novel o/w/o emulsion containing castor oil as the internal oil phase and a fluorocarbon as the external oil phase has been described for pulmonary delivery of the drug[21].

Selection of oil phase can affect various emulsion parameters like yield, release profile, particle size and emulsion stability. The mineral oils give much higher yield than the vegetable oils. In a study, phase volume ratios giving rise to 50% yield were found to be 0.35, 0.29, 0.11, 0.22, and 0.15 for emulsions based on light liquid paraffin, squalane, maize oil and arachis oil, respectively[7]. The release of drug from multiple emulsions is affected by the nature of the oil phase due to difference in partition coefficient of different oil phases for the drug[9,23,24]. W/o/w emulsions prepared with high viscosity oils tend to have larger particle sizes[2,25]. A positive correlation between the interfacial tension at the oil water interface and the internal droplet size has been proposed[23]. Viscous oils produce w/o/w emulsions which are more stable in terms of percentage breakdown[12].

The two-aqueous phases of w/o/w emulsion can be simple aqueous solutions of drugs, buffered solutions, aqueous suspensions of the drug, gelled aqueous phases and aqueous phases containing viscosity enhancers. Release rate can be modified by changing the pH of the two aqueous phases[10,26]. Increase in PH difference between two aqueous phases destabilizes the w/o/w emulsions[12].

Surfactants are used to reduce the interfacial tension at o/w or w/o interface. For formulation of multiple emulsions, at least two stabilizing surfactants are needed. For w/o/w emulsion a lipophilic surfactant is used as primary emulsifier and a hydrophilic surfactant is used as secondary emulsifier while in case of o/w/o emulsion hydrophilic surfactant is required for first emulsification and lipophilic surfactant is required for second emulsification. The optimum surfactant needed to emulsify a given oil can be determined by use of hydrophile lipophile balance (HLB) system[27]. However HLB system does not take into account the effects of surfactant concentration on stability[28]. Florence and Whitehill[11] suggested the optimal HLB value of the primary surfactant to be in the range 2-7 and that of secondary surfactant to be in the range 6-16 for a w/o/w emulsion. Surfactant blends can be used to achieve optimal HLB. Combination of surfactants produces more stable emulsions[29].

Concentration of surfactant also effect the emulsion yield. The use of very low or very high concentrations of surfactants is not advocated because very low concentration may not be able to stabilize the emulsions while use of very high concentration may cause toxicity[11]. Matsumoto *et al.*[1] suggested that concentration of lipophilic surfactant (span 80) required for 90% and more yield was more than 30% w/w of the oil phase but high concentration of hydrophilic surfactant in external aqueous phase of w/o/w emulsion decreased the yield. Also, an excess of lipophilic surfactant can cause the inversion of w/o/w emulsion to simple o/w emulsion[30]. For preparation of w/o/w emulsions, Jager-Lezer *et al.*[31] calculated the minimal lipophilic surfactant concentration needed to saturate the primary w/o interface by taking into account the primary emulsion composition, molecular mass of lipophilic surfactant, average diameter of the internal aqueous droplets and the molecular area occupied by the lipophilic surfactant on the saturated primary interface (determined by interfacial measurements). It was assumed that the oil phase formed a lipophilic surfactant reservoir when surfactant concentration in it was more than the above calculated value. Thus by these calculations optimal surfactant concentration can be found.

Excess of hydrophilic surfactant destabilizes the w/o/w emulsion by solublization of lipophilic surfactant and thereby reducing its concentration at the primary w/o interface[1,30]. Attempts have been made to correlate interfacial film strength with stability of w/o emulsion[32] and w/o/w emulsion[33]. Excess of hydrophilic surfactant reduces the interfacial film strength at the primary w/o interface by co-adsorption at primary interface or by solublization of lipophilic emulsifier in aqueous phase or by both mechanisms[33]. The nature of hydrophilic surfactant also affects the structure of multiple droplets[34]. Jager-Lezer *et al.*[31] showed that swelling capacity of multiple w/o droplets increases with an increase in lipophilic surfactant concentration. These workers have proposed that swelling causes appearance of voids in the hydrophilic surfactant membrane. When an excess of lipophilic surfactant is present in the oil phase, it moves to the o/w interface and fills up the voids created, by droplet swelling, in the hydrophilic surfactant membranes. Thus the swelling capacity of the multiple droplets increases and bursting and subsequent release of markers is decreased or delayed. High swelling capacity also leads to stable multiple emulsions. Geiger *et al.*[35] used a novel micropipette aspiration method to evaluate elastic shear modulus of multiple globules and confirmed the predominant role of lipophilic surfactant during the swelling phase.

Magdassi *et al.*[36] reported that yield and stability of w/o/w emulsions was dependent upon the type of emulsifier and its HLB. It was found that most stable w/o/w emulsions were formed when there was a chemical similarity between the oil phase and the hydrophobic part of the emulsifiers and as well as between the pair of emulsifiers. The most commonly used lipophilic and hydrophilic emulsifiers are Spans and Tweens respectively. These are nonionic type of emulsifiers, having a wide range of HLB, available commercially. Ionic emulsifiers are not used because they can react with the ionic drugs. Moreover, the yield of w/o/w emulsions is better with nonionic type of emulsifiers than the ionic type[1].

Stability of multiple emulsions:

Some of the breakdown pathways that may occur in a multiple emulsion are coalescence of multiple droplets with each other, coalescence of internal droplets, expulsion of internal droplets, and shrinkage of internal droplets due to diffusion of water through the oil phase[11]. The causes for instability of multiple emulsions are migration of emulsifier, osmotic instability and creaming.

Migration of lipophilic emulsifier to the external aque-

ous phase can occur leading to depletion of lipophilic emulsifier in the oil phase and rupture of oil layer with consequent loss of the internal aqueous droplets. Emulsifier migration leads to decrease in effective HLB of the second emulsifier proportional to the concentration of primary emulsifier in the oil phase. The shift of the optimal HLB at various emulsifier concentration is due to existence of free primary emulsifier in the oil phase of primary emulsion. A linear correlation between the optimal HLB, the concentration of second emulgant and the reciprocal of concentration of primary emulsifier was observed. At a fixed concentration of primary emulsifier, the HLB shift is inversely proportional to the concentration of secondary emulsifier. This relationship is helpful in predicting optimal HLB if the two emulsifier concentrations and the required HLB of the oil are known[37]. Inversion of w/o/w emulsion to a simple o/w emulsion can occur if the HLB of the total emulsifier (i.e., the migrated lipophilic emulsifier and the hydrophilic one) approaches the HLB of the oil or if the droplet size becomes too small to hold internal aqueous droplets due to increasing secondary emulsifier concentration[1,38].

Collings[8] reported rapid breakdown of w/o/w emulsions at the site of injection and no prolonged effect of the formation was obtained. It was due to the fact that the osmotic pressure in the external environment (body fluids) was greater than that in the internal aqueous phase of the emulsion. This led to the movement of water from the internal aqueous phase to the external aqueous phase through the oil layer with consequent shrinkage of internal aqueous droplets and rupture of the oil layer. If the osmotic pressure in the internal aqueous phase is higher than that of the external aqueous phase, water may pass from the external phase to the internal aqueous phase leading to swelling of internal aqueous droplets which eventually burst releasing the solutes[31,35]. Since the viscosity of w/o/w emulsion depends on continuous phase viscosity, movement of water across the oil phase due to osmotic effects the viscosity of the system[11,15].

A w/o/w emulsion, upon keeping, may show phase separation due to density difference between the oil phase and the aqueous phase or due to large size of multiple drops. Decreasing the density difference between aqueous and oil phase, increasing the concentration of secondary surfactant or increasing the viscosity of external aqueous phase, can reduce creaming.

Methods to stabilize multiple emulsions:

Interfacial complexation improves the stability of w/o/w emulsions by reducing coalescence of internal aqueous droplets. Interfacial complexation refers to a physical interaction between a nonionic lipophilic surfactant (present in the oil phase) and some macromolecule (eg. bovine serum albumin, gelatin) present in the internal aqueous phase of the w/o/w emulsion[39-42]. This interfacial complex is formed at the primary w/o interface and occurs in form of a complex membrane and develops over a period of time[40,42]. Release of solutes from such system is slow.

Formation of a polymeric gel in the internal or external aqueous phase of w/o/w emulsions renders good stability of these systems[9,43,44]. Gelling agent can be added to the two aqueous phases or the gel may be formed by *in situ* polymerization[43,44] using gamma-radiation to affect cross-linking between molecules of gelling agent present in either of aqueous phase. Gelling affords stability by blocking the coalescence of multiple droplets (by gelling of external aqueous phase) and by blocking coalescence of internal aqueous droplets (by gelling of internal aqueous phase). A viscosity enhancer (like hydroxypropylmethyl cellulose, polyvinyl pyrrolidone, acacia, galatin) can be added to the two aqueous phases of a w/o/w system to prevent creaming and coalescence of multiple droplets[45-47].

Various studies suggest the use of hypertonic inner aqueous phase to reduce (or delay) the separation of aqueous phase from w/o/w emulsions[15-18]. Kawashima *et al.*[18] reported that entrapment percentage of solute in the internal aqueous phase increased with increase in concentration of solute (glucose or sodium chloride) in the internal aqueous phase of w/o/w emulsions. Such emulsions had delayed separation of aqueous phase and lesser decrease in percent entrapped upon storage. Mechanism of stabilization is the thickening of oil membrane. Such emulsions have high viscosity and consequently delayed flocculation and phase separation. Judicious use of osmotic additives in the internal or external aqueous phase, as demanded by the system, can be done to overcome osmotic instability[8]. Adjustment of osmotic imbalance by addition of osmotic agents to the external aqueous phase also leads to retarded release of drugs from the w/o/w emulsions[23,48]. Adjusting the density difference between the oil phase and the aqueous phase can reduce creaming[2]. They added lipiodol ultra fluid to isopropyl myristate in order to obtain a mixture, which has density equal to that of water.

Drug release from multiple emulsions:

Diffusion of solute through the oil layer seems to be the most obvious mechanism for transport of unionized lipid

soluble drugs[11]. Diffusion of unionized drug 5-fluouracil entrapped in inner aqueous phase of w/o/w emulsion across oil phase or through localized thin oil lamellae is the primary transport mechanism[23]. This is supported by the fact that a w/o/w emulsion of 5-fluorouracil, after release of drug, retained multiple character; suggesting release of drug by diffusion. Liquid membrane systems used for treatment of drug overdosage are based on diffusion controlled transport of drug from the external to internal aqueous phase through the oil layer[49]. Drug transport *in vitro* has been found to follow first order kinetics, Fick's law being obeyed.

Mechanisms involving transport of ionized materials through the oil layer have been proposed[11]. One is carriage of water in mixed inverse micelles of hydrophobic and hydrophilic surfactants and second mechanism involves diffusion of water across very thin lamellae of surfactant formed where the oil layer is very thin[50]. Garti *et al.* also proposed micellar transport for water permeability[51]. Ionized materials can thus permeate the oil layer along with the diffusing water. A third possible mechanism involves carrier mediated transport[52]. Additives were also found to be successful carriers for the transportation of glucose[53]. Also solubilization of small amount of internal phase in the intermediate membrane phase may account for transport of very small of materials.

Recently many authors have reported that release of water soluble drugs from w/o/w emulsions occurs by a swelling breakdown behavious[31,35]. When a w/o/w emulsion is placed in a hypotonic media (hypotonic with respect to the internal aqueous phase of the w/o/w emulsion) water moves from the external to the internal aqueous phase due to osmosis. The oil membrane acts as a semipermeable membrane. The internal aqueous droplets (and consequently the multiple droplets) swell and eventually burst thus releasing the solutes.

Brodin and Frank[54] studied the *in vitro* release of naltrexone and thymol from o/w/o emulsions and found that a biphasic drug release pattern was observed. A rapid alpha (a) phase followed by a slower beta (b) phase was observed with naltroxone release. However, at lower drug concentration, only b phase was observed. This biphasic patter is probably due to initial fast release originating from drug leaked in the outermost phase during the preparation of emulsion followed by slower release of drug from the innermost phase of the system.

Florence and Whitehill[11] have suggested release of unionized material from w/o/w multiple emulsion follows first order kinetics. Magdassi and Garti[55] have proposed a kinetic model for release of electrolytes from w/o/w emulsions by considering the internal aqueous phase to be analogous to a dispersed solid in the oil membrane. This system is similar to a polymeric matrix containing the drug in dispersed form. Higuchi has given release kinetics from a polymeric matrix[56]. The model assumed that drug is dissolved from the surface layer of the matrix; when first layer gets exhausted of the drug, drug release from second layer starts. For a matrix of slab geometry, the release fraction is dependent linearly on the square root of time and reciprocal of initial drug concentration. The model for release of dispersed drug from a spherical matrix was shown to be suitable for the release of electrolytes from multiple emulsions[55].

CHARACTERIZATION OF MULTIPLE EMULSIONS

Rheology of multiple emulsions:

Stability assessment can be made by suitable rheologic measurements[18,57-62]. Viscosity changes over time show the volume fraction instability of the dispersed globules and therefore multiple emulsion instability[31]. Elucidation of drug release kinetics can be done by using rheological analysis[57,59,63]. Viscosity measurements in combination with conductivity measurements, can give information about release mechanism from w/o/w emulsions[63]. In one such study w/o/w emulsions with sodium lactate in the internal aqueous phase were prepared and diluted with various concentrations of glucose solutions. The diluted emulsions were subjected to rheological and conductometric analysis. Emulsions diluted with glucose solution of certain concentration, showed no variation in apparent viscosity (suggesting stability of multiple droplets) but showed an increase in conductivity value, revealing release of electrolyte. Thus it was proposed that release was due to diffusion of electrolyte through oil membrane and not due to rupture of oil film[63]. When the w/o/w emulsion was diluted with distilled water, the rheogram showed an increase in apparent viscosity followed by a decrease. This was attributed to initial movement of water from external to internal aqueous phase, leading to swelling of multiple droplets, followed by their rupture.

Rheological behaviour of w/o/w emulsions was studied by Kawashima *et al.*[64] using cone and plate type viscometer. A negative thixotropic behaviour was observed at low shear rates. This negative thixotropic behaviour became more pronounced and the apparent viscosity increased upon increasing the shear rate, prolonging the shear time, or repeating the shear. Further shearing caused a rapid increase in the

shear stress of emulsion and induced phase inversion. This phase inverted emulsion was of w/o type and in a semisolid state. This type of rheological behaviour was attributed to the increase in the volume fraction of the oil droplets by entrapment of water molecules and by coalescence of the oil droplets upon shearing.

Aging conditions can be generated in the multiple emulsions by subjecting them to excessive shearing[57,58]. Such studies can be used to determine the effect of aging upon stability of multiple systems. In addition to stability determination, rheological measurements have cast light on the phenomenon of permeation of solutes and water through oil membrane of w/o/w emulsions[65,66]. In an interesting study Tomita and coworkers[65] studied the viscosity changes in w/o/w emulsion upon dilution with various solutions. It was seen that solutions of urea and potassium thiocyanate increased the viscosity while glucose, calcium chloride, and potassium chloride decreased the viscosity. Based on this observation they suggested that solutes which produced a greater increase in viscosity contributed to a smaller degree to the osmotic pressure of the outer phase i.e., the solutes could more or less permeate the oil layer. The permeability sequence was according to the following order, urea>potassium thiocyanate>potassium chloride > glucose > calcium chloride. This sequence of permeability was found to be in good accordance with the permeability coefficient values[65]. Such studies suggest that water and solutes can permeate the oil membranes of the w/o/w emulsions leading to change in vesicle volume, thereby causing change in emulsion viscosity. Such studies also raise question on validity of the dialysis based methods of yield determination as they are based on the assumption that the solutes detected in the dialysis media originate only from leakage during the preparation procedure of w/o/w emulsions and during dialysis. However if the solute can permeate the oil layer, these is no way to distinguish between the solute diffused (permeated) from the internal aqueous phase and solute leaked out due to destruction.

Compared to w/o/w systems very limited work has been done on rheology of o/w/o systems. The o/w/o multiple emulsions are highly non-Newtonian. The degree of shear thinning in multiple emulsions increases with increase in volume fraction of primary o/w emulsion in o/w/o emulsion. However an increase in viscosity with aging is only marginal in case of o/w/o emulsions[67].

Yield/entrapment efficiency determination:

Yield of a w/o/w multiple emulsion can be defined in terms of number and volume percentage of simple and multiple droplets, and in terms of efficiency of entrapment of an marker molecule in the internal aqueous phase of w/o/w system[7]. Thus the methods for yield analysis can be divided in two groups[7]. First is size analysis technique in which particle size distribution of the system is analysed by a suitable method such as microscopy or coulter counter. The relative proportion of simple and multiple droplets is found[68]. Second is internal phase tracer technique which is based on establishing the entrapment efficiency of a marker in the internal aqueous phase of the w/o/w system. The two groups of methods can not be compared directly as they measure different parameters altogether[7]. The size analysis technique measures merely the physical presence of multiple droplets and not the entrapment efficiency. Thus it is possible that a system may be having good yield in terms of observed multiple droplets but with little marker entrapped in the internal aqueous phase. In contrast, the tracer technique measures only the difference in concentration of marker in the internal and external aqueous phase. It is very much possible to have maximal theoretical marker present in external aqueous phase (i.e., apparent zero yield) and yet many multiple droplets present in the system.

Freshly prepared emulsion placed in a dialysis bag are dialyzed against a suitable volume of dialysis media for a suitable period of time and the quantity of marker migrated to the dialysis media is analysed and the yield/entrapment efficiency of the emulsion is calculated by a suitable equation[1,2,15,18,64,69]. These methods assume that the marker detected in the dialysis media originates only due to leakage during the preparation of emulsion. Migration of marker through the oil phase, during the dialysis process, is taken as negligible. In some methods[7,13] the external aqueous phase is separated from the w/o/w emulsion by a suitable technique (centrifugation). The concentration of marker present in the external aqueous phase is analyzed and yield is calculated. Conductivity measurements of w/o/w emulsions, diluted with water, could give the concentration of (ionic) marker in the diluted external aqueous phase and thus the yield can be calculated[31,70].

Particle size analysis of multiple emulsions:

Methods like optical microscopy and coulter counter can be used for size analysis of the dispersed oil phase[11]. Some of the oil droplets contain small aqueous droplets and this results in bimodal size distribution. The size characteristics of the two types of emulsion particles and their change with time can be resolved using a graphical inflexion method[71].

Size analysis of internal aqueous droplets is relatively difficult and tedious by optical microscopy; although used by various authors[9,26,31]. Moreover small simple drops may pass below the layer of simple droplets to give a false impression of multiple droplets. Reflection of light from the surface of oil droplets[1] and Brownian movement of droplets are other problems. Size analysis of the internal aqueous droplets can be done easily by sophisticated particle dispersion analyzer[2].

A freeze etching method[72] using the electron microscope has been used successfully for size analysis of internal aqueous droplets. But it is an expensive and time consuming method not suitable for routine analysis. In another method[73], based on semipermeable nature of thin oil membranes, the emulsion was exposed to the osmotic gradient provided by electrolyte in the external aqueous phase. Droplet shrinkage resulted from movement of water from internal aqueous phase to external aqueous phase. The rate of shrinkage, related to surface area and the volume of internal aqueous phase was measured by coulter counter. But it was not able to distinguish between a simple and multiple droplets. Granulometric analysis, using particle size analysers, can be used to obtain parameters like volume/surface diameter[31,35].

Stability studies:

Stability assessment of multiple emulsion involves various aspects like determination of particle size, determination of phase separation, measurement of entrapment percentage and viscosity. Measurement of particle size after regular time intervals can give idea about any kind of structural changes in the system. The separation of phases by placing the multiple emulsion in graduated cylinders can be monitored at regular time intervals to detect creaming or sedimentation. Measurement of entrapment percentage at regular intervals can give information about leakage of marker or solute from the internal aqueous phase. Viscosity measurement at regular intervals can be used to detect any structural changes in the multiple emulsions upon storage[18,57]. Kawashima *et al.*[18] suggested that stability of w/o/w emulsions at room temperature can be predicted from the results of accelerated stability tests at high temperature. Aging conditions can also be produced by subjecting the multiple emulsion to shear[57,58].

***In vitro* release studies:**

Release kinetics have been mainly studied by dialyzing the multiple emulsion packed in a dialysis tube against a suitable dissolution media. Some *in vitro* drug release studies for topical[74] and oral[48,75] multiple emulsions have made use of diffusion cell where the emulsion sample is placed in a donor compartment and the drug is released from this compartment to the receptor compartment by diffusion through a dialysis membrane. Conductometric methods based on conductivity measurements can be used to detect release of ionic markers from w/o/w emulsions[30,31,63].

***In vivo* evaluation:**

Using suitable animal models can do *in vivo* characterization of multiple emulsions. Many studies have been done where blood concentration data in rats[3,4,24,42,76,77], rabbits[3,37] and mice[48,75,78] was determined after administration of multiple emulsion. Making use of urinary excretion data in human subjects had performed oral bioavailability studies of isoniazid[79] and nitrofurantoin[80] w/o/w emulsion.

APPLICATIONS OF MULTIPLE EMULSION SYSTEMS

The rationale behind use of multiple emulsions as prolonged and controlled drug delivery systems is that the drug present in the innermost phase has to cross several phases before it is available for absorption from the system. W/o/w emulsions for parentral delivery are more convenient to handle, use, and inject due to lower viscosity of these systems. Many authors have studied the use of multiple emulsion for oral[75,78,81-83], parenteral[3,8,84], and topical[1,63,74,85,86], and ophthalmic[87-89] prolonged release of various drugs. Multiple emulsions have been found to be useful in enhancing lymphatic accumulation of anticancer drug[90-93]. Enhanced lymphatic accumulation of these drugs leads to better anticancer activity. Various workers have proposed the use of w/o/w multiple emulsions in treatment of drug overdosage[49,94,95]. Frankenfeld *et al.*[19] reported *in vitro* removal of salicylates and barbiturates by w/o/w emulsion and observed that the emulsion was capable of rapid uptake of drug *in vitro*.

Liquid membranes have been used for various separation purposes like separating hydrocarbons[96] and treatment of waste water[97]. The liquid membranes are similar to multiple emulsions except that no secondary surfactant is used in these systems. Use of multiple emulsions for taste masking of bitter drugs has been described by some authors[98,99]. W/o/w emulsions have been successfully used as carrier of insulin and bioavailability of insulin from these systems was much improved[76,77]. The cause of improved bioavailability from multiple emulsion system is that the drug contained in the innermost phase is protected from the enzymes present in the GIT. Nowadays multiple emulsion technique is utilized extensively for formulation of microspheres[100,101]. Multiple

emulsions have been utilized for many other uses like as immunological adjuvants and carriers of vaccines[102,103], for immobilization of enzymes[104,105], as cosmetic formulations[106,107], and as food products[108,109].

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