Composition and Preparation of Experimental Intravenous Fat Emulsions

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SUMMARY

Components and procedures used to prepare intravenous fat emulsions for animal experiments are described. These are exemplified by details for making the emulsions containing 7,12-dimethylbenz(a)anthracene used to induce mammary cancer and leukemia.  

INTRODUCTION

An extensive review of the literature concerning composition, preparation, metabolism, and clinical utilization of intravenous fat emulsions has been published by Geyer (2). Of the various preparations proposed for clinical use, a 15% cottonseed oil emulsion stabilized with purified soybean lecithin and Pluronic-F-68 (Lipomul® I.V., The Upjohn Company) (12) was studied most extensively in animals and man. It was found to be well tolerated by animals and humans during short-term administrations, but the possibility of the development of a “long-term reaction” (14) in humans receiving multiple infusions necessitated restrictions in its use (13, 14). Although Lipomul® I.V. is no longer available for use in humans, The Upjohn Company has prepared experimental emulsions for animal use in studies of lipid metabolism and as vehicles for parenteral administration of fat soluble agents. Most notable examples are the emulsions containing the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA), made a popular tool for cancer research by Huggins (6, 7). The present communication details the methods used in preparing DMBA emulsions at The Upjohn Company so that they may be duplicated by other laboratories. With appropriate changes in composition these procedures also apply to other experimental emulsions.

MATERIALS AND METHODS

Composition of Emulsion

The formula for 6 kg of 15% fat emulsion with 5 mg DMBA per gm is given in Table 1. The description of components follows:

Cottonseed Oil. Whereas a variety of oils could be used, a commercially processed cottonseed oil (Wesson® Wesson Sales Co., Fullerton, California) was preferred (15). It has a low color index, low free acidity, and resistance to oxidative changes. It is a uniform product produced in large quantities under clean, controlled conditions, and is available from local markets.

Purified Soybean Lecithin. Purification of soybean phosphatides was necessary to obtain a stabilizer that would not cause granulomatous lesions in organs of animals (4) or depress blood pressure and respiration (5, 12). Quantities of the fraction of soybean phosphatides we used, sufficient for laboratory use, were prepared according to a special procedure (10). Five hundred gm of alcohol-soluble soybean phosphatides (Centrophile SA, Central Soya, Chicago, Illinois) was stirred for 30 min in 5 liters of 95% ethanol. After standing overnight at 20 ± 2°C, the supernatant was siphoned off and stirred 30 min with 1 kg of aluminum oxide. This mixture was allowed to settle about 1 hr, and the supernatant was filtered through an alcohol-washed Seitz filter pad. The clarified solution was concentrated to 70 ± 5% solids content by vacuum distillation at internal temperatures of ≤ 30°C. This yielded about 200 gm of solids which were stable for over 1 year refrigerated in sealed containers. The ethanol solution of purified lecithin was added to the oil of the emulsion on a basis of solids content.

Pluronic F-68. This is a nonionic, water-soluble polyoxyethyleneoxypropylene polymer produced by Wyandotte Chemical Corp., Wyandotte, Michigan. It is metabolically inert (3) and aids formation and physical stabilization of the emulsion (9, 11).

Water. Sterile, pyrogen-free distilled water was used. The resulting emulsion is hypotonic to blood, but where emulsions are to be used as vehicles for drugs administered in small volumes, this is of no consequence. Where isotonicity is important, we recommend addition of dextrose to the emulsion at a final concentration of 4% just prior to administration. Dextrose was added to the emulsions just prior to administration

Table 1

<table>
<thead>
<tr>
<th>Components</th>
<th>% (w/w)</th>
<th>gm/6 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottonseed oil (Wesson®)</td>
<td>15.0</td>
<td>900</td>
</tr>
<tr>
<td>Purified soybean lecithin</td>
<td>1.2</td>
<td>72</td>
</tr>
<tr>
<td>Pluronic F-68</td>
<td>0.3</td>
<td>18</td>
</tr>
<tr>
<td>7,12-Dimethylbenz(a)anthracene</td>
<td>0.5</td>
<td>30</td>
</tr>
<tr>
<td>Water for injection</td>
<td>83.0</td>
<td>4980</td>
</tr>
</tbody>
</table>

Formula for 6 kg of 15% fat emulsion with 5 mg of 7,12-dimethylbenz(a)-anthracene per gm.

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to avoid phosphatide breakdown and/or reactions of phosphatide with the sugar during sterilization (1) or storage (8). We have observed that, after 2—3 years storage at 4°C, emulsions without dextrose had titratable acidities (ml of 0.1 N NaOH have observed that, after 2—3 years storage at 4°C, emulsions in which these solutions were used, decrease in pH when autoclaved (17). These observations suggest that, during heat sterilization, the aqueous dextrose phase of phosphatide-containing emulsions forms products that initiate phosphatide decomposition.

DMBA. 7,12-Dimethylbenz(a)anthracene (Eastman Kodak) was purified1 by chromatography on silica gel (Davidson, grade 950, mesh 60—200, activated by heating overnight at 100°C). A solution of hydrocarbon in the minimum volume of warm benzene was applied to the column (50 times the weight of DMBA) prepared in hexane. Elution with hexane to remove the benzene, then with 5% ether in hexane (v,v) provided pure 7,12-dimethylbenz(a)anthracene.

Preparation of Emulsion

Equipment. Emulsions were produced in a Cherry-Burrell Junior 125 Viscolizer (capacity 125 gallons per hr) equipped with an external cycling system (Chart 1). The homogenizing surfaces (homogenizing valve and plug) were inspected regularly for signs of wear and usually required relapping after 5 hours of use. Prior to emulsion preparation, the equipment was rinsed and checked for leakage by cycling distilled water under pressure, and then drained.

Process. The DMBA was dissolved in the lecithin and oil on a steam bath. The Pluronic F-68 was dissol

Chart 1. Diagram of high-pressure homogenizing system used in preparing i.v. fat emulsions.

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1This method was developed by Dr. R. G. Harvey, The Ben May Laboratory for Cancer Research, The University of Chicago.
to indicate emulsion quality. Generally, ability to evaluate by this method indicates that the bulk of emulsion particles were $\leq 1 \mu$, since emulsions containing large percentages of particles $>1 \mu$ do not transmit light in the hemocytometer.

**DISCUSSION**

The method and materials used to produce emulsions with small particle sizes and remarkable stability have been described. When less satisfactory emulsions are obtained, the difficulty can usually be traced to homogenizing with worn valves or air trapped in the system, cycling completed emulsion under reduced pressure, or failure to minimize evaporation from the surface of the emulsion after sterilization. Although 3- to 6-kg lots were generally prepared by this procedure, the method may be modified for smaller quantities by using a Waring blender to prepare a premix of the water and oil phases, which can be added to the homogenizer and recycled continuously for 4 min at 3000 psi.

Experimental emulsions were also prepared in which DMBA or other related hydrocarbons were incorporated in the preparation. Initially each compound was purified, and solubility in cottonseed oil was determined at room temperature as a guide for proper formulation. After solubilization of each compound in oil with the aid of heat, the emulsion was prepared in the usual manner. Emulsions containing 0.5% DMBA or 0.25% concentrations of 7,8,12-trimethylbenz(a)anthracene, benz(a)anthracene, benzo(a)pyrene, or 3-methylcholanthrene were prepared.

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**REFERENCES**


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