morphine. Since opium contains also other alkali-soluble alkaloids than morphine, these, if present in appreciable quantities, may also be included with the morphine and thus show an apparent higher morphine content. Pharmacopœias applying a correction for "assay-loss" should, as a matter of scientific accuracy and of fairness to the manufacturers of morphine and its derivatives, who consume 90% or more of the total legitimately used opium, take cognizance of the occlusion of by-alkaloids in the morphine and make the necessary correction.

By coincidence of counterbalancing error factors, the U. S. P. assay of opium appears to indicate very closely the true morphine content.

The "total extraction" of the opium which has been practiced in the assay by the several revisions of the U. S. P. is an important point in its favor. It obviates errors in aliquot portions due to the variable amounts of water and insoluble matter in the opium. In the assay method under consideration by the Committee of the League of Nations, published elsewhere, these sources of "inaccuracies" are corrected for by making separate determinations of the water content of the opium, and of the total extractive matter.

Corrections, almost of any kind, are looked upon with disfavor in analytical procedures. They are most uncertain and most undesirable when the corrections involved are of appreciable magnitude.

Assays based on the isolation of the morphine, free from by-alkaloids, through the use of immiscible solvents offer a possible solution of the problem provided they can be worked with reasonably large samples. They should also strive to avoid such aliquots as may introduce any element of error, and, *ipso facto*, should not require an undue length of time.

GELATIN AS A STABILIZING COLLOID FOR OIL IN WATER EMULSION SYSTEMS.*

BY LINWOOD F. TICE.[1]

Various workers have investigated the efficiency of gelatin as an emulsifying agent. Briggs and Schmidt (1) found gelatin to be comparatively inefficient as an emulsifying agent. Clayton (2) reported drop numbers for cottonseed and peanut oils in aqueous gelatin solutions which indicated that gelatin possessed considerable ability to reduce the interfacial tension between oil and water. Holmes and Child (3) studied the effect of added electrolytes upon the emulsion system kerosene, gelatin and water and concluded that the important factor was the conferring of a favorable viscosity to the gelatin solution. Kernot and Knaggs (4) using the Donnan pipette measured the drop numbers of various oils against gelatin solutions. Limburg (5) showed that a lowered p_H favors the adsorption of gelatin around oil globules.

In reviewing the results of these workers it is very difficult for one to reach any definite conclusions as to the exact status of gelatin as a practical emulsifying agent. The following criticism may be advanced concerning these results:

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* Scientific Section, A. Ph. A., Portland meeting, 1935.

[1] Department of Research, Philadelphia College of Pharmacy and Science. Investigation conducted in behalf of the Edible Gelatin Manufacturers' Research Society of America, Inc.

I. The use of the drop number of an oil against some solution, although of considerable indicative value, cannot be taken as conclusive evidence in regard to the emulsion stabilizing ability of such a solution.

II. In no case was really effective emulsification of commonly used oils, *e. g.*, cod liver oil, heavy mineral oil, etc., actually accomplished. Such emulsions as were produced were of theoretical interest but they possessed no practical value.

III. Such inefficient methods of dispersion were employed that there exists considerable doubt as to the real meaning of the results.

It must be realized that a substance may be an excellent stabilizer in an emulsion system, effectively reducing the interfacial tension between the two phases, but at the same time due to its physical properties it may not favor the easy dispersion of the phase to be dispersed. Consequently, using crude methods of dispersion one is apt to obtain misleading results as to the true stabilizing power of an emulsifying agent.

When a highly efficient device which insures an initially highly disperse emulsion is employed then the real ability of a substance to stabilize the system can be determined.

The object of this investigation was to determine the efficiency of gelatin as an emulsion stabilizing colloid, the specifications under which it is most effective for such use, and lastly, if possible, to submit practical working formulas in which gelatin might be employed as the emulsifying agent.

COLLECTION OF MATERIALS.

Samples of every type and grade of gelatin were obtained from several of the largest manufacturers of this product. All the data concerning the method of manufacture and the control reports were submitted with each sample.

The next step was the investigation of emulsifying devices in order to obtain the most efficient unit available. The importance of such an investigation has already been explained, namely, to eliminate all possibility of misleading results due to inefficient mechanical treatment.

Several colloid mills were examined and the degree of dispersion accomplished by each noted. Then a laboratory homogenizer manufactured by Manton-Gaulin was studied. The results were as follows: Among the colloid mills there is a wide difference in their relative efficiencies. The homogenizer, however, for liquid emulsions provided the best degree of dispersion and the greatest uniformity of product. It is true that colloid mills are not as cumbersome and are more generally applicable for other uses than the homogenizer, but for liquid emulsions they did not prove as satisfactory.

Furthermore, gelatin being strongly adsorbed and probably preferentially so at a water/air interface, the homogenizer which almost entirely excludes air was thought to be more satisfactory for the preparation of emulsions containing gelatin.

Having now decided upon the method of dispersion to be used and having available every possible type of gelatin, the actual experimental work was begun.

EXPERIMENTAL PART.

The experimental method chosen was the preparation of emulsions of heavy liquid petrolatum containing in each case 50 per cent of oil, the dispersion medium being aqueous gelatin solutions in which the type of gelatin, its concentration and the p_H of the solution were the factors varied. In each case the mixed phases were homogenized at a pressure of approximately 3500 lbs. The results are tabulated below.

	Concentration.	p_H.	Condition of Emulsion.
A—Bone Gelatin, 200 Bloom.			
1	0.5%	5.8	Poor
2	1.0%	5.8	" , gel formation
3	1.0%	4.0	" " "
4	1.0%	3.0	" slight gel formation
5	1.0%	7.0	" " " "
B—Calfskin Gelatin, 200 Bloom.			
1	0.5%	6.1	Poor
2	1.0%	6.1	" , gel formation
3	1.0%	4.0	" " "
4	1.0%	3.0	" slight gel formation
5	1.0%	7.0	" " " "
C—Porkskin Gelatin, 250 Bloom.			
1	0.5%	4.6	Fair
2	1.0%	4.6	Good, gel formation
3	1.0%	4.0	" " "
4	1.0%	3.0	Excellent
5	1.0%	7.0	Poor, gel "
D—Porkskin Gelatin, Isoelectric.			
1	0.5%	8.0	Poor, slight gel formation
2	1.0%	8.0	" gel formation
E—Bone Gelatin, Isoelectric.			
1	0.5%	4.7	Poor, slight gel formation
2	1.0%	4.7	" gel formation

From the above results it will be seen that the behavior in the case of C—4 was outstandingly good. These results were checked and it was found that a porkskin gelatin at a p_H of 3 gave an excellent, highly dispersed emulsion.

Upon the basis of these results the following explanation was derived which seems to provide a sound theoretical reasoning on which basis the above behavior can be interpreted.

Bone and calfskin gelatin are derived following a preliminary liming treatment of the precursor. The excess lime is removed by washing and acid washing followed by partial hydrolysis and extraction of the highly purified collagen by heating with water. In the case of porkskin gelatin, fresh frozen porkskins are treated with acid in the initial stage, followed by partial hydrolysis and extraction of the collagen. It is already known (6) that gelatin derived from alkali-treated precursors and gelatin obtained from an acid-treated precursor have quite different isoelectric points, the former having an isoelectric point at a p_H of 4.7 and the latter having an isoelectric point falling at a p_H of approximately 8.

As the p_H of a gelatin solution is lowered from its isoelectric point the gelatin becomes positively charged, such charge increasing with lower p_H levels, according to the equilibrium positive gelatin $\rightleftarrows$ isoelectric gelatin $\rightleftarrows$ negative gelatin.

Oil globules dispersed in water have been shown to be negatively charged which is as would be expected inasmuch as the dielectric constant of oil is lower than that of water.

Therefore, in order to encourage the adsorption of gelatin at an oil/water interface the greater the charge the more readily should such adsorption take place.

Starting with a gelatin from a limed precursor whose isoelectric point lies at 4.7 it will readily be seen that in order to confer on it highly positive properties a much lower p_H is needed than would be required by a gelatin from an acid-treated precursor having an isoelectric point at a p_H of 8. This seems to be a logical explanation of the superiority of porkskin gelatin in stabilizing an emulsion over bone or calfskin gelatin at the p_H of 3.

It was evident that if this reasoning were correct bone and calfskin gelatin at a still lower p_H, say approximately a p_H of 1, should make possible equally satisfactory emulsions as those given by porkskin gelatin at the higher p_H. This was definitely borne out experimentally.

Another difficulty using ordinary bone or calfskin gelatin is that, at ordinary p_H levels, if sufficient gelatin is used to provide the necessary concentration gelation of the product results. With porkskin gelatin this tendency is very much reduced, and at a p_H of 3 gelation of even 1% solutions is almost entirely prevented. The reason for this difference is explained by the fact that the degree of hydration of gelatin is proportional to the differential between its p_H and that at its isoelectric point. The degree of hydration of a porkskin gelatin at a p_H of 3 would consequently be expected to be considerably more than that present in a bone or calfskin gelatin at that same p_H.

Although emulsification is quite efficient at a p_H of 1 with bone and calfskin gelatin, for most purposes at least this p_H level contributes too great an acidity to the product and for emulsions to be taken internally the use of porkskin gelatin at a p_H of 3 is recommended.

Porkskin gelatin as sold upon the market has a p_H of 4.0–4.6. Consequently, an adjustment of the p_H level is necessary before it may be used in the preparation of such emulsions. In the above experiments such adjustments were made on the basis of a graph on which the ordinates were the cc. of tenth-normal hydrochloric acid added per 100 cc. of 1% gelatin and the abscissæ the resulting p_H levels. Between the extremes p_H 4.6 and p_H 3 the plotted points produced practically a straight line and it was found that with various lots of gelatin of the same type, knowing their initial p_H, it was possible within reasonable limits to calculate by proportion the required amount of acid that should be employed.

It was thought, however, that for the practical worker it would be much easier to adjust the p_H with tartaric acid. Tartaric acid being a quite pure and uniform crystalline substance, the addition could then be made on a weight basis. A graph was prepared in which the milligrams of tartaric acid added per 100 cc. of 1% gelatin was represented as the ordinates, the abscissæ being the observed p_H levels of such solutions. Such a graph is illustrated (Fig. 1).

Emulsions were made using the proper amount of tartaric acid to provide a p_H of 3. Such emulsions, however, seemed considerably more acid to the taste than similar emulsions made with gelatin whose p_H was adjusted with hydrochloric acid. The reason for this may be that some tartness lies in the undissociated tartaric acid molecules, such tartness being an inherent property of these molecules to the taste. Fortunately, it was found that at a p_H of 3.2, where only three-fifths as much tartaric acid was required, emulsification was satisfactory and the gelation tendency sufficiently depressed by the added liquefying effect of the tartrate that gelation did not occur. Consequently, if the p_H is adjusted by the use of hydrochloric acid the level should be 3; if tartaric acid is used a level of 3.2 is most satisfactory.

Alcohol, in moderate amounts, sugar and glycerin were found to be compatible with gelatin in emulsion formulas. In fact, the last two seemed to actually exert a favorable influence on the resulting product.

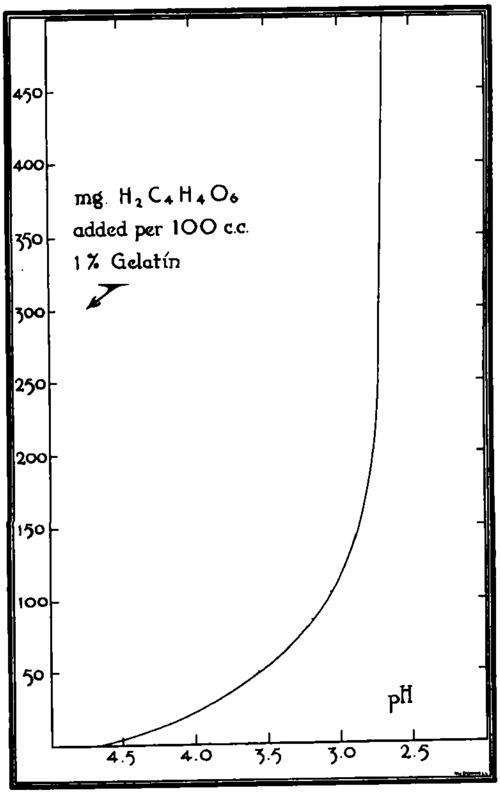

Fig. 1.

Various samples of experimental emulsions were aged to determine their stability; others were placed in a refrigerator and a third lot were subjected to oven temperatures of approximately 45° C.

The result of several months' aging at room temperatures is a very slight creaming and a gradual loss in viscosity. The extent of creaming is considerably less than many comparable commercial emulsions. The gradual loss in viscosity was not unexpected as, under such conditions, a slow hydrolysis of the gelatin protein is of course unavoidable. The degree of dispersion and condition of emulsification remained excellent.

Those emulsions placed in the refrigerator became somewhat thick and viscous but no actual solidification took place. It was in some cases necessary to shake the bottle before pouring, due to the formation of a thin surface film which, however, was easily broken on shaking.

A very interesting phenomenon was observed with emulsions placed in the oven at 45° C. When such emulsions were made with hot gelatin solutions and subsequently cooled they were perfectly stable. On the other hand, when made with cold gelatin solutions the emulsions after being kept in the oven showed a slight separation of oil, which separation, however, was not continuous. There are two possible explanations seen for this—first, it may be that the sol $\rightleftarrows$ gel change due to change in temperature may cause a certain amount of dispersed oil to separate when made in the cold and then warmed, or secondly, the cold gelatin solution may, due to its greater viscosity, entrap air, this entrapped air at the same time enclosing oil globules. Upon heating, the air being expelled a certain small amount of unemulsified oil is liberated. This latter explanation seems the more probable of the two. At any rate, such a defect is overcome by using a hot gelatin solution in producing the emulsion.

Realizing that a slow hydrolysis of gelatin might in time entirely destroy its stabilizing influence, the following experiment was carried out: A 5% solution of gelatin at a p_H of 3 was heated at 100° C. for 3 hours. At the end of this time it had lost all power of gelation and it was, when cold, quite limpid, yet it still quite effectively retained its power to stabilize an emulsion containing 50 per cent mineral oil. Of course, the hydrolysis had not yet completely formed the underlying aminoacids, although doubtlessly it was considerably progressed. This experiment gave good evidence that although hydrolysis would unavoidably lessen the viscosity it should not be expected to disrupt the emulsion system through total loss of stabilizing influence.

It may be mentioned here that loss in viscosity, although it accelerates slightly the rate of creaming, is not nearly as undesirable as an increase in viscosity upon aging. In the latter case, actual solidification may occur whereupon pouring may become impossible.

A comparison of relative efficiencies of low and high Bloom gelatins showed conclusively that the higher Bloom gelatins are to be preferred in emulsion work. Consequently, porkskin gelatin, for example, was selected having a Bloom rating of at least 250 Gm. No difficulty should be experienced in obtaining such a Bloom strength gelatin of the porkskin type, although with bone and calfskin gelatin a product of this high a Bloom rating is not usually marketed.

There are several advantages to be obtained by the use of gelatin as an emulsifying agent.

I. It is an excellent stabilizer.

II. It possesses unusual economy due to both its low cost and the small amount required. It was calculated that for one gallon of emulsion the cost of gelatin required would be less than three cents.

III. For emulsions to be administered internally it greatly reduces the calorific intake as compared with acacia. Furthermore, being very easily digestible it is less disturbing in cases of gastro-intestinal disorders.

IV. In technical emulsions it eliminates the presence of the, in many cases, undesirable gum and permits the preparation of highly fluid yet slightly acid emulsions.

The only possible disadvantages encountered are first, the necessity of a homogenizer, and second, the gradual loss in viscosity of the finished product.

The first disadvantage is meaningless on a large scale as most manufacturing organizations possess such equipment. On a small scale there are now available numerous quite inexpensive hand homogenizers (7) which make possible the use of gelatin, *e. g.*, by the prescription pharmacist.

It may be mentioned that the new United States Pharmacopœia has its text so worded that gelatin may be used in official emulsions replacing acacia.

PRACTICAL APPLICATIONS.

Some formulas using gelatin as the sole emulsifying agent are presented in order to illustrate the practicability of its use.

A.

I	Gelatin (0.6% porkskin, 250 Bloom)	6.0 Gm.
	Tartaric acid *or*	0.450 Gm.*
	(Hydrochloric acid *q s.*)	
	Syrup	100.0 cc.
	Water *q. s.*	
	To make	440.0 cc.
II	Vanillin	0.035 Gm.
	Alcohol	60.0 cc.
III	Heavy mineral oil	500.0 cc.
	To make about	1000.0 cc.

Add the gelatin and tartaric acid to about 300 cc. of cold water, allow to stand a few minutes, then heat until dissolved. Add the syrup and finally enough water to make 440 cc. (I). While still quite warm add II, then III, and mix well avoiding the inclusion of air. Finally, homogenize and bottle. Homogenization should be repeated if necessary until all the oil is completely dispersed.

Such an emulsion conforms to the new U. S. P. XI monograph for Emulsion of Liquid Petrolatum.

B.

I	Gelatin (0.5% porkskin, 250 Bloom)	5.0 Gm.
	Tartaric acid *or*	0.375 Gm.*
	(Hydrochloric acid *q. s.*)	
	Methyl parahydroxy-benzoate	2.0 Gm.
	Water *q. s.*	
	To make	500.0 cc.
II	"Lemon Oil"	250.0 cc.
	Light mineral oil	250.0 cc.
	To make about	1000.0 cc.

The directions here are similar to those in "A." Methyl parahydroxy-benzoate replaces the alcohol as a preservative. Such a formula provides an excellent furniture polish emulsion.

In preserving emulsions made with gelatin the only organisms that can develop at such a p_H are the molds and yeasts. All proteolytic bacteria are inactivated by the acidity. Consequently, the problem of preservation is no more complicated than that arising from the use of any carbohydrate emulsifier as, for example, acacia.

SUMMARY.

I. Gelatin is a very efficient stabilizing colloid for oil-in-water emulsion systems.

II. The important factors to be considered in connection with gelatin for this purpose are first, the preliminary treatment received by its precursor (which determines its isoelectric point) and second, the p_H of the solution to be used.

III. Gelatin from acid-treated precursors, having an isoelectric point at p_H 8, requires a p_H of approximately 3 to effectively stabilize an emulsion, whereas gelatin

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* The amount of tartaric acid may vary slightly depending upon the gelatin used. If hydrochloric acid is used, the amount necessary to give a p_H of 3 must be determined by experiment. If a thinner emulsion is desired 5 Gm. of gelatin may be used instead of 6 Gm.

from alkali-treated precursors, having an isoelectric point at p_H 4.7, requires a p_H of approximately 1.

IV. The advantages of gelatin for use in emulsions are enumerated.

V. The efficiency of several colloid mills as compared with that of a homogenizer in preparing liquid emulsions is reported.

VI. Practical formulas and directions for the use of gelatin in emulsions are presented.

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A NOTE ON THE ACTION OF ALKALIES AND ALKALI SALTS ON ANTIPYRINE.*,[1,2]

BY LOYD E. HARRIS AND ERCELL D. TEBOW.

A prescription that called for a solution of antipyrine in a concentrated solution of potassium citrate was brought to the laboratory for an explanation. A colorless liquid separated which disappeared on dilution of the mixture with water. Or, on standing, it gradually changed to a crystalline substance. A search of the literature gave no information as to what was happening, so the problem was investigated in the laboratory.

EXPERIMENTAL.

A few grams of the liquid precipitate were obtained by adding antipyrine to a saturated aqueous solution of potassium citrate. This was separated by using a separatory funnel. Crystals began forming after standing a few hours and in about 48 hours the mass was completely crystallized. The crystals resembled antipyrine in physical appearance and the melting point was found to be 111° C. which is the same as the U. S. P. compound. A mixed melting point with antipyrine did not cause any change. The U. S. P. X color tests were positive, thus, further indicating that the crystals were antipyrine.

A saturated alcoholic solution of picric acid was added to a boiling aqueous solution of the immiscible liquid. Almost immediately, long yellow crystals began to appear. After washing with water and drying, their melting point was determined to be 188° C.; antipyrine picrate was prepared in a similar manner and its melting point was the same.

A second portion of the aqueous solution of the immiscible liquid was made acid with hydrochloric acid (no change was apparent in the dilution used) and then sodium nitrite was added. The green precipitate, which formed, was separated by filtration, washed with water and then dried. The melting point was 200° C. and corresponds to the melting point of nitroso antipyrine, prepared in the laboratory.

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* Scientific Section, A. PH. A., Portland meeting, 1935.

[1] An abstract from a Thesis submitted by Ercell Dale Tebow to the Graduate Faculty, University of Oklahoma, in partial fulfilment of the requirements for the degree of M.S. in Pharmacy, 1933.

[2] From the Pharmacy Laboratory, School of Pharmacy, University of Oklahoma.